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No. 6.

AWARD OF THE BOYLE MEDAL TO PROFESSOR J. DOYLE, D.Sc.

Report of the Committee of Science and its Industrial Applications recommending the award.*

The Committee of Science has recommended the award of the Boyle Medal to Professor Joseph Doyle in recognition of the high value of his work on the Reproduction of the Conifers, which has been described in a brilliant series of papers contributed to the Society's Scientific Proceedings.

His earliest publication in these Proceedings dates from 1915. It describes how the grafting of a bud on a leaf stalk converts this petiole into a stem. By Professor Doyle's method of experimentation not only does the petiole take on the functions of a stem, supporting new leaves and supplying them with water and nutritive substances from the soil, but it is fundamentally changed in form and internal structure, and acquires the physical and biological characteristics of a stem. His next publication in the Society's Proceedings contained new observations on *Larix leptolepis*. In this he showed the presence of a mechanism in this larch for receiving the pollen, which had been known hitherto only in the genus *Pseudotsuga*. Further, he noted and recorded the absence of air bladders in the pollen of the larch, a characteristic which up till then the larches were supposed to share with the pines and firs. In this work he seems to have come under the charm of the great group of conifers, and from that time forward he has made these majestic trees his especial study.

Cold-resistance in plants had been attributed to the accumulation of pentosans in their tissues. Doyle, however, showed that in the conifers, which afforded many examples of cold-resistant trees, there is no relation between pentosan-content and cold-hardiness. This research opened the way for investigations on seasonal changes in coniferous leaves with special reference to enzymes and starch formation, and to the presence and rôle of catalase in the metabolism of conifers. His work in this direction was published in the Royal Irish Academy's Proceedings.

Then came a series of papers on what may be spoken of as the concealed, or cryptic, life of the Gymnosperms. In revealing its recondite secrets, Professor Doyle's work has been an outstanding contribution. The structural changes and processes which form the subjects of his investigations take place

* The medal was awarded by the Council on June 18, 1942, and presented to Professor Doyle by the President at the Stated General Meeting of the Society on November 12, 1942.

only in the terminations of the high branches of the trees, so that the acquisition of adequate sequences of material is unusually laborious and difficult, and much of that acquired only by effort and pertinacity, has to be rejected as not illustrating the sought-for stage. In 1929, with W. T. Saxton, he gave a detailed account of the peculiar male gametophyte, the formation of archegonia, the development of the pro-embryo, and the earliest stages of the embryo in *Athrotaxis selaginoides*—a Tasmanian tree rarely cultivated in this country, but which may be seen at Kilmacurragh, Co. Wicklow.

That was followed by a study of the pollination of *Fitzroya* and an account of the early stages of its male gametophyte and of some abnormal cones of this genus. Like *Athrotaxis* it is antipodean, and native in Chili and Tasmania. It is very rarely found in collections in the British Isles, and only those forming female gametophytes had hitherto been examined. Information that a pollen-bearing specimen belonging to a sympathetic owner, was available in Worcestershire, made this investigation possible, and evidence was obtained clearing up this tree's life-history, and shedding light on the morphology of the coniferous strobilus. Ever since the results of these investigations have appeared, a continuous stream of publications, published in our Society's Proceedings, has come from members of Professor Doyle's school, under his guidance and inspiration.

These memoirs throw new light and give much new information on this cryptic life in the genera, *Saxegothea* (Patagonia), *Pinus, Cedrus, Tsuga, Pseudotsuga, Callitris* (Africa, Madagascar, Australia, New Caledonia) and the great *Sequoia*—the giant trees of California. Many interesting facts and novel ideas are recorded in these writings which cannot be referred to here. One may be mentioned: the bladders of the pollen grains of *Pinus* not alone act as wings, but also, as the grains are immersed in the pollination drop, function as floats which raise the pollen grain up through the micropyle, and orient the development of the emerging pollen-tube. Thus these pollen plants are microscopic organisms which begin life as aircraft, are later water-borne, and finally become internal parasites.

It is pleasant to think that we may expect much more from Professor Doyle. His work is still in full swing. We note in this connection that one of his contributions has only just been published, and another is listed for reading at our next Scientific Meeting.

No. 7.

POLLINATION IN *TSUGA PATTONIANA* AND IN SPECIES OF *ABIES* AND *PICEA*.

By JOSEPH DOYLE and ANN KANE,
University College, Dublin.

(Plates 2 and 3.)

[Read November 24, 1942. Published separately March 15, 1943.]

Observations were published some years ago (Doyle and O'Leary, 1935 a, b) on pollen reception, and micropyle structure in relation to it, in certain members of the Pinaceae (then referred to as the Abietineae). These observations were made on certain representative species of the genera *Cedrus, Larix, Pinus, Pseudotsuga,* and *Tsuga.* The present paper supplements these observations, with some along the same lines on certain species of *Abies* and *Picea* and on an additional important species of *Tsuga*; and extends still further the range of types of pollination mechanism within the family. To avoid nomenclature confusion the specific names here used are those in Pilger (1926), without, however, implying any opinion as to their correctness.

TSUGA PATTONIANA.

Doyle and O'Leary (1935 b) have already described the pollination phenomena in *Tsuga heterophylla* and *Ts. dumosa* (= *Brunoniana*). In both pollen falls on the cone scales, the tubes growing along these to reach the micropyles. These two species are included by Pilger (1926) in the section *Eutsuga,* and probably all the species belonging to this section would show the same behaviour, as all seem to share the character of wingless pollen grains. There is, however, at least one other true species in which the grains *are* winged, viz., *Tsuga Pattoniana.* It differs also in certain structural features from most of the members of the genus, and is frequently separated in the section *Hesperopeuce.* It was obviously of interest to determine the pollen behaviour in this case.

It is still uncertain whether *Tsuga Pattoniana* is the only species with winged grains. Thus there have recently been described certain new species of *Tsuga* which, being amphistomatic, may properly belong to the section

Hesperopeuce. These species are *Ts. longibracteata* and *Ts. crassifolia,* the latter possibly identical with *Ts. Pattoniana.* Details of these and other species can be found in the recent revision of the genus by Flous (1936). No data, however, are anywhere given of the nature of the pollen grains; and no fertile specimens, living or herbarium, are available here. There is also *Ts. Jeffreyi* Henry. This species was for a long time described as a variety of *Ts. Pattoniana,* but is now commonly given specific rank, and is considered to be a fertile hybrid—*Ts. Pattoniana* × *heterophylla.* Some living specimens are growing in English gardens, but in this case also it proved impossible to locate or obtain fertile material for personal examination nor could any record be found of the nature of its pollen grains. A cross between species, one (*heterophylla*) with winged grains, and one (*Pattoniana*) without wings, would repay examination, especially in view of the fact that in *Ts. Pattoniana* the structure of the ovule and the pollen behaviour are different from those of *Ts. heterophylla,* as the description below brings out. It is hoped to examine this point if occasion offers.

It is of further interest in this connection that Wodehouse (1935) in his book on pollen grains seems to have overlooked the existence of *Ts. Pattoniana,* and considers wingless pollen grains characteristic of the whole genus. "Apparently," he says (p. 267), "there is in existence to-day no intermediate form between that of the wingless *Tsuga* grain and the winged form of the Abietineae." He refers, however, to fossil species of *Tsuga* from the Tertiary which bear rudimentary bladders, and concludes, probably quite justly, that the present form of the wingless *Tsuga* grain is derived from an ancestral winged type.

Specimens of *Ts. Pattoniana,* though not common, are to be found in many gardens in Ireland and Britain, but cones are rarely produced. No coning tree could be traced in Ireland during the period in which the observations recorded in this paper were being made, and unsuccessful application was made to many gardens in Britain where specimens were known to exist. We greatly appreciate the fact that, at all the places written to, the gardeners or owners made diligent search for the young cones, which are small and inconspicuous. Happily, however, a tree in Westonbirt carried a few cones in 1936 and in 1939. Our best thanks are due to Mr. W. J. Mitchell, the head gardener, for his trouble in locating, securing, and forwarding, in excellent condition, a number of cones at suitable intervals. These cones, though relatively few in number, were sufficient for our purpose.

Pollination takes place in *Ts. Pattoniana* early in the third week in May. The young ovules, at this time, are shown in adaxial view in Pl. **2**, 1, and in abaxial view in Pl. **2**, 2. The micropyle area differs markedly from that of the two species already described (Doyle and O'Leary, 1935 b, Pl. **4**, 1). The integument here shows a lobed flaring expansion adaxially, while on the abaxial side a broad slit runs down a short way to expose the tip of the nucellus. The shape, degree of lobing, and extent of spread of the flare, vary, as is shown

in the two ovules in Pl. 2, 1. The ovule on the left shows a commoner type, but the flap may be more splayed in some cases. This flap is composed of typical succulent and semi-translucent stigmatic tissue, and is undoubtedly a stigmatic expansion which partially blocks the passages between the cone scales. When pollination is effected the grains are found adhering to various parts of the stigmatic area—on the edge, well in towards the nucellus, and also on the outer surface. In spite of the shape of the ovule, grains are rarely found actually on the nucellus. Comparatively few remain lodged on the scales, in marked contrast to the behaviour in the other species.

The condition about five weeks later is shown in Pl. 2, 3. The dissected ovule on the right, in which the archegonia are faintly indicated, shows that development has proceeded rapidly. Actually, in the collection of this date, stages were found from male cells just before fertilization to two-tiered proembryos. Little change has taken place in the stigmatic area, except that it has become flattened on the shoulders of the ovule as this presses, in growth, against the axis and the neighbouring scales; or part of it may, in some cases, be turned over across the micropyle. Shortly after pollination growth of the nucellus pushed its tip a little higher, so that at the stage here shown, particularly in the dissected ovule on the right, it fits into the actual micropyle opening like a plug. Its upper cells take on something of the appearance of a stigmatic tissue in preparation for the reception of pollen tubes. A receptive zone in the upper part of the nucellus is fairly common, even in species in which the pollen lodges directly on it, but the development here is more distinct and comparable to that in the araucarians; the nucellar flare in *Saxegothaea* is an extreme expression of this. The figure also shows that the pollen has germinated where it fell, the tubes growing, fairly directly, to reach and pierce this receptive area. Lodgment on the stigmatic surface of the micropylar expansion is, however, not essential for pollen germination. In the left of the figure a couple of grains which lodged near, but not on, the stigmatic area are shown forming tubes which successfully reached the nucellus. In the case of grains further away, the one on the extreme left for example, although germination may take place, the growth of the pollen tube is not so definite in direction. The general tendency of growth is along the scale, or the surface of the ovule, towards the cone axis, but no tubes have ever been seen to reach the nucellus.

Tsuga Pattoniana thus shows still another type of pollination mechanism in the Pinaceae. It differs from that described for other species of *Tsuga* mainly in (*a*) the presence of wings on the grain, (*b*) the development of a stigmatic flare round the micropyle, and (*c*) the poorer growth power of the pollen tubes.

ABIES.

Pollination phenomena in this genus have been referred to by Strasburger (1871) for the common fir and for *A. balsamea*. He says that ovular structure

in *Abies* closely resembles that in *Larix*. The edge of the micropyle is said to be expanded into a one-sided helmet-shaped outgrowth, which only differs from that in *Larix* "durch einen, oft schwachen, mittleren Einschnitt." The pollen grains "werden in das Innere eingesogen," and come to lie in a depression on top of the nucellus. Exactly how this happens is not described, nor is any specific mention made of a fluid secretion. He further states that the edges of the integument do not later dry up or curl inwards, but can be seen for a long time unchanged in form and position, the micropyle remaining open. This account, while in a way correct, is on the whole misleading and inaccurate, and is probably the result of too rapid and cursory observations. Goebel (1933) also mentions *Abies*, but very briefly. He refers micropylar form to three classes—the tubular, the pincers, and the stigmatic types. The tubular type is the common one, as seen in the Cupressaceae, Taxaceae, etc., and is associated with the secretion of the well known pollination drop. The stigmatic type is that of *Larix* and *Pseudotsuga*. The pincers type, he says, is probably characteristic of most Abietineae (Pinaceae as used in this paper), and is shown by *Pinus* and *Abies*. This statement is also incorrect, as *Abies* in structure and behaviour is quite different from *Pinus*.

Several species of *Abies* were observed, and all showed the same general features. Certain species showed minor differences, but as *A. Nordmanniana*, being more accessible, was more closely observed than the others, its description is taken as a type, only brief reference being made to some of the others.

(A.) *ABIES NORDMANNIANA*. Pollen is shed on the average between the 5th and 12th of May. The ovulate cones are already fairly large, 5 cm. by 2 cm., although the ovuliferous scales are still quite small. At this stage the most characteristic feature of the ovule is the flange-like expansion of the integument around the micropyle. It is shallowly notched at places, with, in some cases, a deeper notch on the side towards the median axis of the scale. This wide flare is not so striking, although obvious enough when viewed from the adaxial side, as shown in Pl. 2, 4, because the micropylar neck bends downwards a little, the cone itself standing rigidly erect. The flare is seen best when the cone is cut across and viewed from below. The whole flare is then exposed facing downwards and slightly outwards, as shown in Pl. 1, 5. The young nucellus lies slightly deep, but may be seen if the lie of ovule is such that the micropyle can be viewed vertically. It is shown in the ovule on the left, but appears there higher than it really is. The flange is stigmatic in function, and grains can be found on it at any part, but it cannot be as adhesive all over as other stigmatic structures, since the grains fall away from its periphery rather easily. The pollen is thus more commonly found well down towards the micropylar opening, as if the flange functioned rather like a conducting funnel. This is shown in Pl. 2, 5 and 6. The latter drawing is one of an ovule dissected to show the relatively deep position of the nucellus, and the lie of *some* of the grains. Pollen, of course, may be found much higher up, and sometimes lower down, occasionally a grain has even been seen

practically lodged on the nucellus itself. The grains are winged and float well in water. It was thought at first that the position of such grains in the micropyle might be associated with a fluid secretion, somewhat as in *Pinus*. Considerable attention was given to this point, particularly as a fluid secretion was actually seen in the micropyle in occasional ovules. It gradually became clear that any such secretion was not a normal feature. It occurred rarely, showed no tendency to withdraw into the tissue, and could become so thick and mucilaginous that pollen grains could not move in it. The secretion, when it did occur, seemed to be associated with degeneracy in some ovules and in others with local punctures made by a large aphid which often infested the cones. Punctures made anywhere, even on the scales, produced similar drops of secretion from the turgid tissues. This account has been held up to permit the examination of material over several seasons in order to decide this point. It may be further noted here that, in general, the wings or bladders of the pollen in *Abies* are relatively small in proportion to the size of the grain (cf. also Wodehouse, 1935, p. 263).

Hutchinson (1915) has already referred to a very interesting feature in the development of *Abies* based on *A. balsamea*. The pollen grains lie dormant for four or five weeks, but, when germination has begun, growth of the tubes followed by fertilization is astonishingly rapid. "Although the rate is difficult to determine, it is believed that the passage time of the pollen tube does not exceed two days, and probably may be measured in hours." This seems to be a unique phenomenon in conifers, although almost certainly a characteristic of all species of *Abies*. In *A. Nordmanniana* the pollen dormancy is extended to seven or eight weeks, germination not taking place in the grains until some time in the last week of June. In the meantime the cones, scales, and ovules have increased considerably in size. At pollination the cone was approximately 5 cm. long, the scales 3·5 mm. wide, and the ovule, including the flare, 1·4 mm. long; but now the corresponding measurements would be roughly 15 cm., 3 cm., and 0·8 to 1 cm. The micropylar flare is little changed, and can still be clearly seen as a small appendage at the top of the ovule. Apart from increase in size the only marked changes are that the original bend in the neck of the ovule becomes more distinct, the tissues of the neck thicken, particularly on the adaxial side, and, principally due to pressure against the axis as the ovule swells, the adaxial side of the flare is folded over the micropylar opening. The micropyle thus forms a short inclined tube. The nucellus, which develops a shallow depression at its summit, is very closely approached to the lowermost grains, or may even make contact with them in some cases. It has probably grown up a little to this position, although only very slight growth would be needed. All these features are shown in Pl. 2, 7, which is drawn, rather diagrammatically, from a sectioned ovule. Descriptions of intermediate stages and of the form changes of the whole ovule, as well as many other points of structure not essential to the consideration of pollen behaviour, are omitted for reasons obvious at the time of writing (1942).

The gametophyte, which in mid-June was still only in the free-nucleate

stage, is now (the last week of June) almost ready for fertilization. The archegonia are well developed; only the cutting-off of the ventral canal nucleus and the subsequent rapid maturing have to take place, although the pollen grains have not yet germinated. The succeeding days are the critical days. The actual dates of pollen germination and of fertilization probably vary a little from year to year and from tree to tree. Thus in 1936 no grains had germinated by June 30th, but on July 5th stages from fertilization to tiered proembryos were to be found. On which day in July germination first began was not determined, but it may well have been July 3rd or 4th. On the other hand in 1939 pollen grains had germinated in many ovules by June 30th, although fertilization had not taken place in any one examined. It is hoped to examine this point more accurately in the near future; the general outlines of the phenomena are, however, clear enough. In relation to this a further small change takes place in the nucellus. At some time early in these critical days the tip of the nucellus elongates a little, pushing up in a sharp wedge form among the pollen grains. Possibly the bend on the micropylar neck determines the wedge shape, but it results in bringing the nucellar depression in contact with some of the grains if contact has not already been made. Pl. **2**, 8 shows the appearance at this stage. For clarity only one grain is drawn on the depression, but many grains may be found on it. Pl. **2**, 9 shows a face view of the nucellar tip as seen on a plane at right angles to that of the last figure. Other pollen grains very close to but not in actual contact with the nucellus may also germinate, sending down short tubes which again penetrate the nucellus through the now oblique depression. In Pl. **2**, 10 and 11 the apex of the nucellus is shown in side and face view, respectively, with such a short tube growing in. Shortly after this the continued thickening of the neck and shoulders of the ovule encroach on the nucellar tip, crushing it and closing the ovule. By August it shows merely as a brown crushed lamella with the remnants of the grains and pollen tubes squeezed flat in it.

Reference to the behaviour earlier described for *Cedrus* (Doyle and O'Leary, 1935 b) shows a certain broad similarity to that here described for *Abies*. Pollen is caught in both on a spreading stigmatic flare, and upward growth of the nucellus brings it close to the grains, final contact being made by a special apical extension. In *Cedrus*, however, the form of the micropylar area is different, the nucellar function is more dominant, and the pollen tubes grow at a more normal rate. *Abies* thus can be counted as showing still another type of pollination mechanism, which is, however, closely related to that of *Cedrus*.

Strasburger's (1871) statements that no marked changes take place in the micropylar area, and that the micropyle remains open long after pollination, are thus correct; but any comparison with *Larix* is so far from the facts that it is hard to see how any such comparison could ever have been made. Likewise Goebel's (1933) inclusion of *Pinus* and *Abies* in the same pollination class is inadmissible.

(B.) OTHER SPECIES OF *ABIES*. The young ovules in *A. homolepis*, *A. koreana*, *A. nobilis*, and *A. Webbiana* are on the whole similar to those of *A. Nordmanniana*. In *A. homolepis* the body of the ovule projects a little beyond the scale and continues into a much longer micropylar neck, which is very sharply bent downwards almost at right angles. The nucellus is thus deeply sunk and must be inaccessible to air-borne pollen. Unfortunately, as the tree available bore very little pollen, the details of the behaviour could not be followed, but they would probably merely illustrate an extreme example of the type already described. If opportunity offers it is hoped to follow this point also.

A. koreana, however, was followed almost as fully as *A. Nordmanniana*, from which it differs slightly. The micropylar neck is a little longer and slightly more bent, so that, as the ovule swells, its anterior end becomes flattened against the cone axis, and the adaxial side of the micropylar area gets closely pressed against the abaxial side. The micropylar canal is thus much longer; the pollen grains caught by the flare are, except those at the extreme edge, more definitely enclosed; and those grains on the adaxial side of the flare are brought nearer to the nucellar tip, occasionally even being pressed into it. The general appearance in section, at a date about midway between pollination and fertilization, is shown in Pl. 3, 12. The flat anterior end at right angles to the side is very characteristic. Pollen grains may be found on the nucellus or anywhere along the canal and even out at the integument edge. Just before pollen germination the nucellus presses obliquely into the canal, as in *A. Nordmanniana*, but in *A. koreana* the grains at a distance germinate more freely. They have been found germinating from the outer edge of the micropylar canal, though the tubes from these probably do not reach the nucellus. Pollination takes place in the third week of April, and fertilization about mid-June or a little earlier.

Although the behaviour throughout the genus *Abies* is probably fairly uniform, it would seem advisable, in view of the differences, inconsiderable though they be, shown by the few species examined, that a fuller survey of variations within the genus should be made. Some of the species might well exhibit behaviour or structure closer perhaps to that of *Cedrus* or *Tsuga Pattoniana* than to the *Abies* type here described.

PICEA.

Pollination phenomena in this genus have been referred to by Strasburger (1871) and by Scott and Brooks (1937). Strasburger says that *Picea vulgaris* (= *P. excelsa*) resembles *Pinus* in the structure of the ovule and in the possession of two long prolongations of the micropylar edge. Between these the pollen grains pass to reach the rather deep depression at the top of the nucellus; but how this is achieved is not suggested. As far as it goes this account is correct, but it is obviously rather inadequate. Scott and Brooks, on the other hand, give no account of the structure of the integument around the

micropyle, but make the following statement (p. 287): "The integument opens widely, leaving an open passage through the micropyle leading down to the apex of the nucellus. At the same time a small quantity of fluid is secreted within the micropyle on the surface of the nucellus. Some of the pollen grains carried by the wind pass between the ovuliferous scales, and come to rest on the edge of the integument. Another change now takes place; the micropyle closes, the lips of the integument bending inwards and bringing the pollen grains into contact with the top of the nucellus, to which they adhere owing to the liquid which has been secreted." This account is correct, although inadequate, in respect of a fluid secretion, but the latter part is not based on any species of *Picea* available, and, in so far as it purports to describe the behaviour of the common spruce, is definitely wrong.

Several species of *Picea* were available. The account here given describes the behaviour in the first place in *P. excelsa* and then in P. *orientalis*, as these two show marked divergence in their pollination mechanism. A few notes on some other species of *Picea* are added.

(A.) Behaviour in *P. excelsa*. The behaviour in this species resembles that already described for *Pinus* by Doyle and O'Leary (1935 a), to which paper further reference may be made if necessary. The appearance is shown in Pl. 3, 13 of the whole ovule as seen in adaxial view, and in Pl. 3, 14 of the micropylar area as seen in a cone cut across and viewed from below. The neck of the ovule is a little longer than in *Pinus silvestris*, and the integument is more evenly two-lipped than in that species. The view in Pl. 3, 14 brings out (cf. also Plate 2, 5 for *Abies*) the manner in which the expanded integument areas project into the spaces between the scales to catch the air-borne pollen, whose movements in the air currents are to a large extent determined by the form and lie of the scales, as well as by the shape and direction of the cavities formed between them. This aspect of pollination in such cones as these is important, but has been so well described by Strasburger (1871) and others of the older authors referred to by him that no further comment is needed on it here. The edge of the integument, on the side away from the scale axis, rather evenly joins up the bases of the two stigmatic flaps, but on the inner side the edge is interrupted by a definite slit somewhat shorter than that in *Pinus*. This is also shown by Pl. 3, 15, in which the ovule is drawn in a more strictly abaxial view. The nucellus is low and does not show without dissection, but this feature is seen in Pl. 2, 16, drawn from an ovule dissected shortly after pollination. The stigmatic flaps in nature are usually a little closer together than appears in Pl. 3, 14. They have been splayed out a little in the drawing to emphasize their form and appearance; but, otherwise, these ovules show the common type. In some cases the slit is a little deeper, or the stigmatic flaps may be narrower and drawn out into longer processes, in which cases the ovules resemble those of *Pinus* somewhat more closely, although always quite distinct.

As in *Pinus* pollen is caught on the stigmatic flaps. A fluid is secreted, filling the space above the nucellus and welling out beyond the micropyle as a

large drop held by the two flaps and its own surface tension. The pollen wets easily and passes into the fluid, which is then fairly rapidly reabsorbed. The wings of the grains are relatively large, and orientate the floating pollen as in *Pinus*. The top of the nucellus shows a marked depression, the Einsenkung referred to by Strasburger (1871). This is developed before pollination, but becomes deeper after the deposition of the grains as is shown in Pl. 3, 16. The edges of the depression may later grow up a little, partly embedding the pollen, the whole giving the impression of a small pollen chamber. As the grains are never found elsewhere than in the depression, it may be suggested that the reabsorption of the fluid is a function of this region.

Unfortunately it was not possible to keep the common spruce under the same continued observation as the common pine. Young trees with readily accessible cones were not available, and in older specimens cones are commonly carried towards the top of the tree, perhaps 60 ft. up or higher. Also young pine shoots carrying cones keep for a long time quite fresh in water. Indeed in the case of the pine the mechanism was first suggested from many laboratory observations before it was put to the test in the open. Spruce cones visibly deteriorate in less than a day under such conditions, so that observations were more rushed and less satisfactory, but we are quite satisfied that in general the mechanism is similar in both cases. As far as could be judged, however, from the more limited data available from laboratory and field it is probable that the action in *Picea excelsa* is not quite so precise as in *Pinus silvestris*. Thus the secretion may exude at any time once the ovule is mature; it may be found during the day hours, and is thus not a strictly night phenomenon. The exudation also is a more massive drop, and, if secretion takes place before pollination, the pollen may be more frequently caught in it than is the case in the pine. The reabsorption, while rapid, is less spectacular, requiring probably twice as long, while in some cases it is not quite complete, so that a little of it may be left to dry out on the nucellus, or to be very slowly dehydrated by absorption, leaving the grains embedded in a small sticky mass.

Strasburger's (1871) account, therefore, is correct but inadequate. The folding in of the integument, described by Scott and Brooks (1937), does not seem, however, to have any foundation in fact. Actually, after pollination, the stigmatic flaps come closer together, perhaps bending round one another a little, but gradually drying up and fading. Closure of the micropyle is effected by ingrowth and division of the cells of the neck which thickens as in *Pinus*.

Reference was made in the paper on *Pinus* (Doyle and O'Leary, 1935 a) to the fact that most of the drawings in the older authors of the common species in the Pinaceae showed little detail of micropylar structure. It is worth recording that the drawing by Richard (1826, Pl. 15, 4) of *Picea excelsa* is very satisfactory. It is here reproduced as a text-figure, and, though simpler, obviously closely resembles the drawings of this species in this paper. We are indebted to M. Y. Orr, F.L.S., of the Royal Botanic Gardens, Edinburgh, who kindly arranged to have the drawing copied for us from the original work.

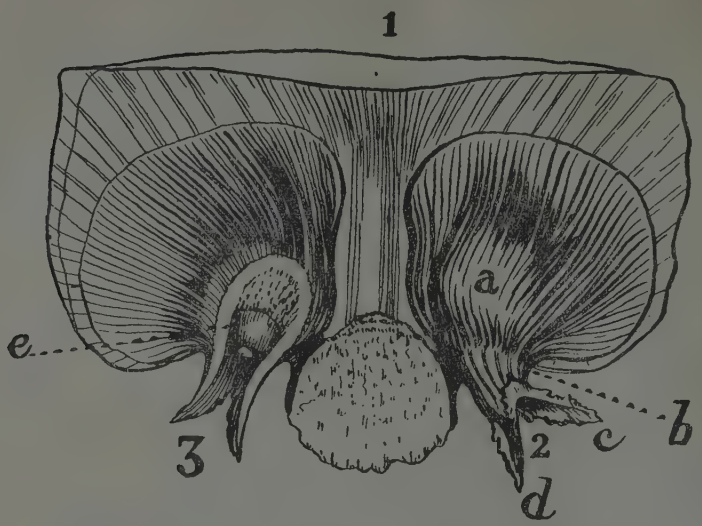

Fig. 1.—*Picea excelsa.* Scale and ovules at pollination (after Richard, 1826).

(B.) Behaviour in *PICEA ORIENTALIS.* This species shows a characteristic which, so far as is known, is unique in the genus *Picea.* The ovulate cones leave the bud inverted, and, when mature for pollination, hang more or less vertically downward—Pl. 3, 18. In other species of *Picea* the cones stand erect when young, only turning down later as they grow older, and this is usually given as a character of the genus—thus Pilger (1926) "♀ Blüten aufrecht." Although *P. orientalis* is a fairly common tree, introduced into Britain as far back as 1839 and represented by good specimens in most collections, no record of this erect position of the young cones has yet been found in any systematic work.

This inverted lie of the cone at pollination bears directly on the pollination mechanism in this species. It was pointed out in the paper on the pollination of *Pinus* (Doyle and O'Leary, 1935 a) that the *erect* position of its young cones may be related to the flotation effect of the wings of the grain. In such cones the ovules are inverted, nearly though not quite vertically, so that the pollen floats up the fluid in the micropylar canal with the germination furrow facing the nucellus. The erect position of the young cones of *Picea excelsa* may have the same relation. If so it is to be expected that pollination behaviour in *Picea orientalis* should, in some important respects, differ from that in *P. excelsa.*

The structure of the integument round the micropyle is shown in adaxial view in Pl. 3, 19. The ovule is rounded, contracting fairly abruptly to a longish neck which is continued as two stigmatic flaps. There is a short slit on the abaxial side. These flaps differ from those in *Picea excelsa* in being much thicker and more succulent, especially at their base. As a result the micropylar

opening is more slit-like than circular. This is well seen looking down on the ovules when the cone is cut across, and is shown in Pl. 3, 20, though here also the flaps have been splayed out a little. The micropylar slit sometimes looks as if closed by the basal swelling of the two lips, but usually a small central area remains more open.

The pollen also shows a special character. Most winged grains, as in *Pinus*, other species of *Picea, Cedrus, Abies,* etc., float in water; wingless grains, such as those of *Larix, Cupressus, Taxus,* etc., sink; but *Picea orientalis* has winged grains which, nevertheless, sink. Its grains are shown in Pl. 3, 21, for comparison with those of *P. excelsa* in Pl. 3, 17. It can be seen that the grains of the former are smaller on the whole, and have relatively much smaller wings or bladders. When blown on to water the grains first float on the surface, and are not quite instantaneously wetted. When wet most of them gradually sink, but the factors determining this need further investigation. There is, however, some evidence to show that watery fluids can penetrate the wing bladders, which become more translucent as they fill with water, the grains thus losing their buoyancy rapidly. This, however, is a property of fresh pollen only; pollen, dried and kept for some time, may float for quite long periods, up to 48 hours in some cases. Again it is hoped to examine this problem more fully, but for the present it suffices that up to 90 per cent. of the fresh grains do sink.

Pollen is shed usually in the second week of May, and is caught by the stigmatic flaps. As in the case of *Pinus silvestris* and *Picea excelsa* the young ovules are in a position to catch pollen before they are fully mature, although the interval may be short. As pollen is plentiful, this early reception is common, although naturally there may be considerable variation depending on the position of the cones in relation to the liberated pollen and the like. When mature, however, the ovule of *Picea orientalis* also secretes a fairly copious fluid, which wells out through the micropyle and lies between the stigmatic flaps. Since the cones are inverted the ovules, and particularly their micropylar parts, are pointing upwards. The pollen grains caught on the stigmatic surfaces are wetted and drawn under the surface of the fluid. They float there for a few minutes, and then sink, passing through the small central channel to reach the neighbourhood of the nucellus. Retraction of the exudate follows, but slowly, needing an hour or more instead of the 10 or 20 minutes taken by the common pine or spruce. That the retraction, slow though it be, is definitely due to the presence of pollen is shown by the fact that the exudation may be continued for a couple of days in unpollinated ovules under identical laboratory conditions. In the laboratory, also, if too many grains are introduced into the fluid some may jam in the narrow opening, and these have often been seen to germinate there. This is quite rare in the field, but has been seen occasionally; such tubes make little progress. Commonly, however, when many grains are bunched on the nucellar top several are not in contact with it, they may be several diameters away. These germinate and successfully reach the nucellus. A dissected ovule with pollen on the nucellus is very similar to one of *Picea*

excelsa, and Pl. 3, 16 would do equally well for *P. orientalis.* Germination of the pollen is rapid, and in both species well developed pollen tubes can be dissected out within a week after pollination.

The behaviour in *Picea orientalis* adds another to the pollination types in the *Pinaceae.*

(C.) Other species of *PICEA.* It cannot be said without a complete survey of the genus whether the type shown in *P. orientalis* is found in any other species, but it is definitely not shown by any available. The following species, at least, in addition to *P. excelsa,* have young cones erect and pollen which floats in water for 24 hours or longer:—*P. alba, brachytila, likiangensis, omorica, polita, rubra, sinensis, sitchensis,* and *Wilsoni.* A fluid exudate reabsorbed after pollination has been observed in the following:—*P. brachytila, omorica, likiangensis,* and *sitchensis.* Presumably all spruces with young cones erect resemble *P. excelsa* more or less closely in their behaviour; but many minor differences exist, and each varying structure may have its own detailed effect on the pollen behaviour. Thus the micropylar form in *P. omorica* and *P. sitchensis* resembles that in *P. excelsa,* but in the Sitka spruce the body of the ovule lies more horizontally, the two stigmatic extensions pointing downwards and markedly at right angles to the plane of the ovule. They are longer and thinner than in the common spruce. In this species the retraction of the fluid after pollination was rapid until, in its withdrawal, it reached the level of the rim of the micropyle; the subsequent much slower reabsorption was, however, not properly studied. In *P. brachytila* the whole ovule is straight, with no flexion of the flaps, and lies at about 45° to the vertical. Only the two flaps, and frequently only part of these, project beyond the scale. A wide micropylar opening is left between the stigmatic extensions, and into this the nucellus has grown at pollination time, completely blocking and even extending a little beyond the micropyle rim. Such an ovule is drawn in adaxial view in Pl. 3, 22; and in Pl. 3, 23 is shown the arrangement seen in the cone cut across and viewed from below. The pollen grains ultimately lodge on the shallow depression there shown, although the grains are not outlined in the figure. Reabsorption of the exudate was quite rapid in this species, taking only about ten minutes. If unpollinated ovules were kept for 24 hours, the exudation drop became steadily larger, but still reacted to the inclusion of pollen. If left still longer before introducing pollen no reaction or only a feeble one was obtained. The same phenomenon has been observed in *Pinus.*

Clearly then there is a fair degree of variation within the genus, and a proper examination of a larger number of species might yield interesting results, especially perhaps among those of the more recent introductions from China, whose systematy is still unsettled. Thus among specimens in the Botanic Gardens at Glasnevin, all included under *Picea likiangensis* and its varieties, there are possibly two different types of ovular structure. Presentation of further details on these and other points already raised in this paper must await the opportunity of fuller observations.

PSEUDOLARIX AND KETELEERIA.

No coning specimen of *Keteleeria* was available. Accessible coning material of *Pseudolarix* was also not available, and, from material sent from Kew, it was clear that without readily accessible material which could be kept under regular personal observation no final conclusions could be made on this genus. The young ovule resembles in general the *Abies* type, and pollen can later be found on the nucellus, but no suggestion can yet be made as to how it reaches this position. It is hoped that some other botanist, to whom coning specimens of these two genera are available, will help to complete these observations on pollination behaviour in the Pinaceae.

Certain general conclusions could be drawn from the data given in this and the preceding papers. It is proposed, however, to deal with these conclusions in a separate later communication.

Summary.

1. Observations are recorded on ovular structure in relation to pollen reception and pollen behaviour in certain members of the Pinaceae not already described in earlier papers.

2. Pollination behaviour in most species of *Picea*, except *P. orientalis*, is of the type earlier described for *Pinus*, although some variation in detail is shown in different species.

3. In *Picea orientalis* the cones hang vertically downwards at pollination. The pollen grains sink in the pollination fluid, even though winged, and thus reach the nucellus of the more-or-less upright ovules. The wings of the grain are relatively smaller, and the stigmatic part of the integument more developed than in other species of *Picea*.

4. In *Abies* no fluid is secreted at pollination. Pollen is held deep in a funnel-shaped stigmatic expansion around the micropyle. The nucellus grows up to make contact with the grains, which then germinate after lying dormant for 5 to 8 weeks. Again, some variation is shown in different species.

5. In *Tsuga Pattoniana* the pollen grains are winged, and its pollination mechanism differs from that earlier described for other species of *Tsuga*. Pollen is caught anywhere on the surface of a spreading micropylar flare, the tubes growing over the stigmatic surface to enter the nucellus tip, which is freely exposed.

6. The conclusions which may be drawn from the data given in this and preceding papers will be discussed elsewhere.

Explanation of Plates.

[All drawings made by Ann Kane. Unless otherwise stated all × 18 approx.]

Plate 2.

1–3. *Tsuga Pattoniana*. 1. Adaxial view of ovules at pollination. May 17. 2. Abaxial view of same. 3. Adaxial view of two ovules about 5 weeks

later to show pollen tube growth, the nature of the stigmatic zone, and the nucellar tip. June 20.

4–11. *Abies Nordmanniana.* **4.** Adaxial view of ovule at pollination. May 8. **5.** Ovules as seen in cross-section of the cone to show nature of the stigmatic micropyle area. May 8. **6.** Ovule dissected to show position of the nucellus and some of the grains. May 8. **7.** Ovule as seen in median section at later date to show position of some of the dormant grains and apex of nucellus. June 30. **8.** Ovule as seen in median section a few days later to show oblique upgrowth of nucellar tip. July 5. **9.** Another view of the same stage in plane at right angles to last fig. **10** and **11.** Side and face view of nucellus at same stage to show growth of short tubes from grains very close to it. (Figs. 7–11 rather diagrammatic.)

Plate 3.

12. *Abies koreana.* Ovule as seen in median section shortly before pollen tube growth to show form of micropylar canal and position of nucellus. May 26.

13–17. *Picea excelsa.* **13.** Adaxial view of ovule at pollination. May 15. **14.** Micropylar region of ovules of same stage as seen in cross-section of cone. **15.** Same in abaxial view. **16.** Ovule dissected to show position of nucellar apex with grains in the depression. May 22. **17.** Pollen grains × 60.

18–21. *Picea orientalis.* **18.** Cone at pollination hanging down. May 8. Natural size. **19.** Adaxial view of ovule. May 8. **20.** Micropylar stigmatic region as seen in cross-section of cone. May 8. **21.** Pollen grains × 60.

22, 23. *Picea brachytila.* **22.** Adaxial view of ovule to show position relative to scale, the form of the stigmatic projections and the slightly protruding nucellus. May 23. **23.** Micropylar region at same stage as seen in cross-section of cone.

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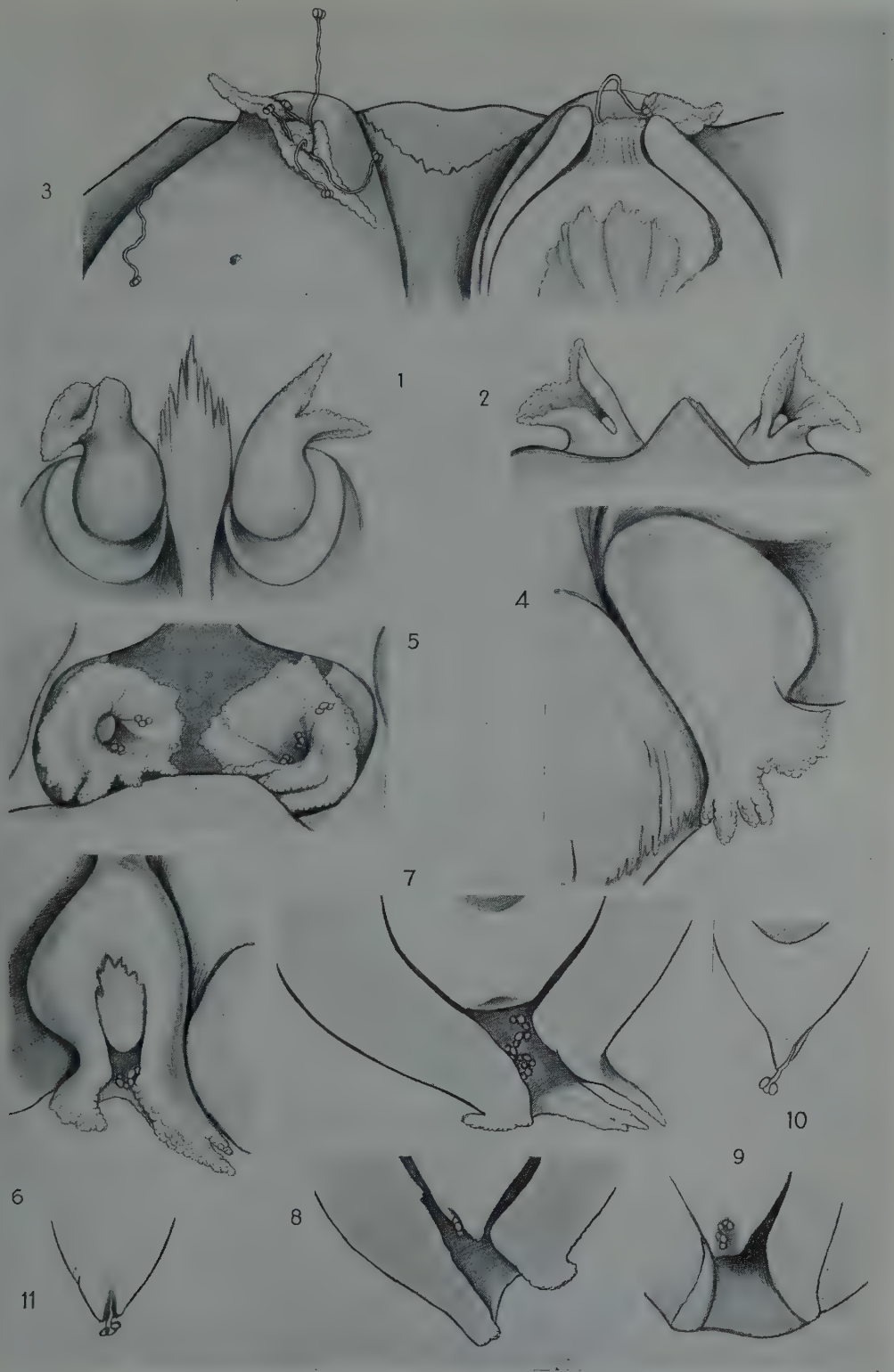
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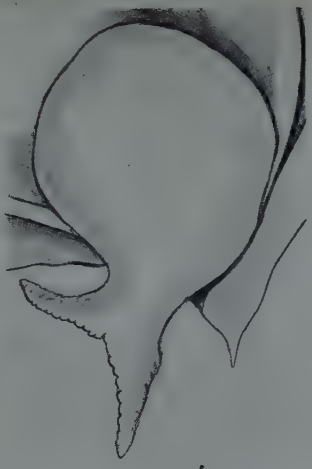

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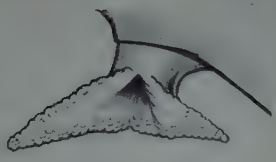

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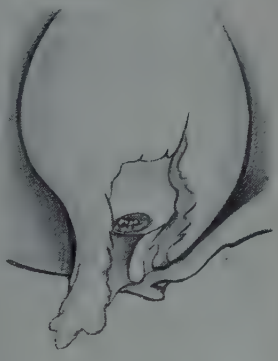

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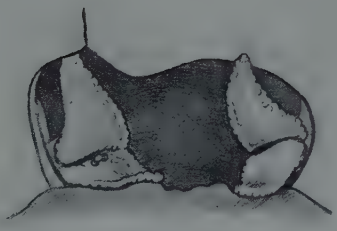

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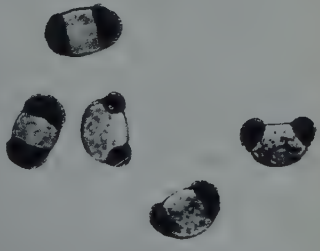

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No. 8.

THE CHEMICAL CONSTITUENTS OF LICHENS FOUND IN IRELAND.—*LECANORA PARELLA* ACH.—THE CONSTITUTION OF VARIOLARIC ACID.

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The first mention in the literature of the examination of the lichen *Lecanora parella* appears in a paper by Schunck (Annalen., 1845, **53**, 257). Schunck isolated from the lichen lecanoric acid, a new acid which he called parellic acid, and some fatty material. It would appear, however, from the investigations of Hesse (J. Prak. Chem., 1906, **73**, 157) that Schunck was mistaken in the identification of his lichen, and that what he had in hand was in fact *Pertusaria lactea*. Nyl., a lichen which in the past has been wrongly classified by botanists as a form of *Lecanora parella*. Both Hesse (*loc. cit.*) and Zopf (Annalen., 1902, **321**, 40) examined the lichen *Pertusaria lactea*. The former found in it lecanoric acid, an acid m.p. 289° C. which he called ochrolechiaic acid, as he first isolated it (J. Prak. Chem., 1902 (2), **65**, 561) from genuine *Lecanora* (*Ochrolechia*) *parella*, and which is undoubtedly identical with the so-called parellic acid of Schunck, and traces of a third acid, which Hesse calls parellic acid, and which we now know to be identical with psoromic acid first isolated by Spica from *Squamaria crassa* (Gazzetta, **12**, 431). Zopf (*loc. cit.*) isolated from the lichen lecanoric acid and an acid m.p. 283° C. which he calls variolaric acid, as the lichen was formerly called *Variolaria lactea*. Zopf states (Ann., 1904, **338**, 58) that variolaric acid and ochrolechiaic acid are identical, though he at first confused it (Ann., **284**, 128) with the psoromic acid of Spica. Hesse (J. Prak. Chem., 1902, **65**, 561) examined genuine *Lecanora parella*, and found no trace of lecanoric acid, but ochrolechiaic acid was present in small amount.

The present investigation of the Irish lichen which was identified for us by Dr. P. O'Connor, Natural History Section, National Museum, confirms this observation of Hesse: in addition we have identified mannitol as the sugar alcohol present. It is an optional matter as to whether the acid in *Lecanora parella* is called ochrolechiaic or variolaric acid; we prefer to adopt the latter term.

The properties of variolaric acid were first described (as parellic acid) by Schunck (*loc. cit.*), who noted its acid reaction towards litmus, and that it

crystallises with water of crystallisation, the amount varying with the conditions. He noted that it could be regenerated from solution in cold caustic potash, but that warming with aqueous potash produced a change, a precipitate being formed only slowly on acidification; he also noted that boiling with barium hydroxide gave a precipitate of barium carbonate, a barium salt remaining in solution which on acidification and extraction with ether gave a product which, like the original acid, gave a violet coloration with ferric chloride. Schunck also prepared the copper and lead salts of variolaric acid, and analysed the original acid, and its hydrates as well as its lead salt. Zopf (Ann., 1902, **321**, 40) amplifies Schunck's description of the properties of variolaric acid. He states that it melts at 285° C. with browning and gas evolution; that it is soluble with difficulty in ether, chloroform, and benzol, and with some difficulty in boiling alcohol; that it gives a colourless solution in sodium or potassium hydroxide, sodium carbonate, or ammonia; that it is not so readily soluble in sodium bicarbonate, and that it gives a colourless solution in sulphuric acid which turns a sherry colour on warming. Zopf also says that in solution in cold potash it is decomposed with evolution of carbon dioxide, and that the acid formed in the process melts at 252° C. and is readily soluble in alcohol, gives a violet reaction with ferric chloride, and an orange red, not blood red, colour with bleaching powder. Hesse (J. Prak. Chem., 1902, **65**, 561) confirms the observations of Schunck and Zopf, and notes that the acid is less soluble in alcohol than psoromic acid; Hesse, however, states (J. Prak. Chem., 1906, **73**, 1152) that the transformation of the acid on standing in potash solution is *not* accompanied by liberation of carbon dioxide, and in this respect our own results support Hesse. Hesse named the transformation product ochric acid, and describes it as melting at 230° C. with evolution of carbon dioxide. Hesse also states that ochric acid with bleaching powder gives a yellow colour destroyed by excess of bleach, a violet colour with ferric chloride, and, with sulphuric acid, a solution turning gradually yellow—statements which our observations support.

For variolaric acid Schunck found for the material dried at 110° C. C = 60·70 per cent.; 61·84 per cent.: H = 3·36; 3·42, and proposes the formula $C_{21}H_{14}O_9$. Hesse, on the other hand, gives the figures C = 62·54: H = 3·64, and proposes the formula $C_{22}H_{14}O_9$. We confirm that variolaric acid contains no methoxyl, but our analytical figures differ from those of Schunck and Hesse. We obtained for the material dried in vacuo at 105° C., C = 61·2; 61·3: H = 3·38 and 3·39. The material was not sufficiently soluble in camphor for a molecular weight determination. We propose the formula $C_{16}H_{10}O_7$. (C = 61·15: H = 3·39.) The alternative formula $C_{18}H_{12}O_8$ (C = 60·67; H = 3·37) is less in accordance with the reactions of the acid though it is close to psoromic acid, $C_{18}H_{14}8_8$, with which variolaric acid has, as regards its reactions, so much in common. When variolaric acid was dissolved in 10 per cent. aqueous potash and allowed to stand for a few hours, a product was obtained m.p. 221–223° C. (evolution of carbon dioxide) which was evidently the ochric acid of Hesse, and produced by the hydration of variolaric acid. The analytical data show that the product was $C_{16}H_{12}O_8$.

When variolaric was heated in 50 per cent. aqueous potash for 5 hours two products were obtained: one of these was soluble in sodium bicarbonate and had m.p. 195° C. with gas evolution; the other insoluble in sodium bicarbonate, but soluble in sodium hydroxide had m.p. 194–6° C. with no gas evolution. The product soluble in sodium bicarbonate was $C_{15}H_{14}O_7$, while the product insoluble in sodium bicarbonate was $C_{14}H_{14}O_5$. The reactions of variolaric acid with alkalies are thus in accordance with the equations:

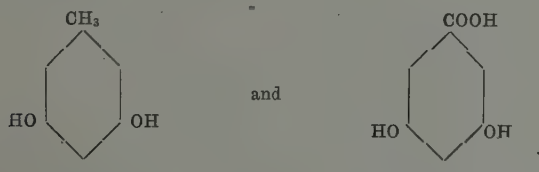
$$C_{16}H_{10}O_7 + H_2O = C_{16}H_{12}O_8$$
$$C_{16}H_{10}O_7 + 2H_2O - CO_2 = C_{15}H_{14}O_7$$
$$C_{16}H_{10}O_7 + 2H_2O - 2CO_2 = C_{14}H_{14}O_5.$$

These reactions recall the corresponding type of reactions in the case of psoromic acid as elucidated by Asahina and Hayashi (Ber., 1933, **66**, 1023).

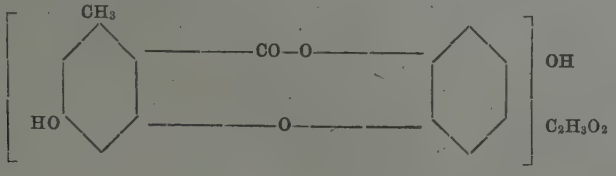
$$C_{18}H_{14}O_8 + H_2O = C_{18}H_{16}O_9 \text{ (paric acid)},$$
$$C_{18}H_{14}O_8 + H_2O - CO_2 = C_{17}H_{16}O_7 \text{ (parellinic acid)}.$$

Variolaric acid forms a diacetyl derivative and with diazomethane a dimethyl derivative which is not demethylated on prolonged boiling with potash. These results indicate the presence of two hydroxyl groups, both aromatic, and the absence of a carboxylic acid group. Treated with methyl alcoholic potash variolaric acid adds on methyl alcohol forming an ester, and the resulting product on methylation with diazomethane forms a compound which now contains four methoxyl groups. These results are explained by the presence of a lactone bridge in variolaric acid, which on opening with methyl alcoholic potash forms an ester, and liberates a new phenolic group.

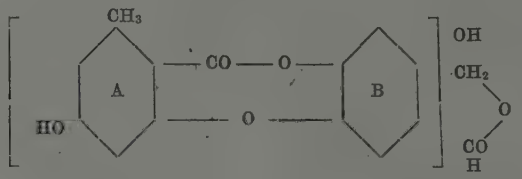
When variolaric acid is fused with potash two products are formed, namely orcin and α-resorcylic acid.



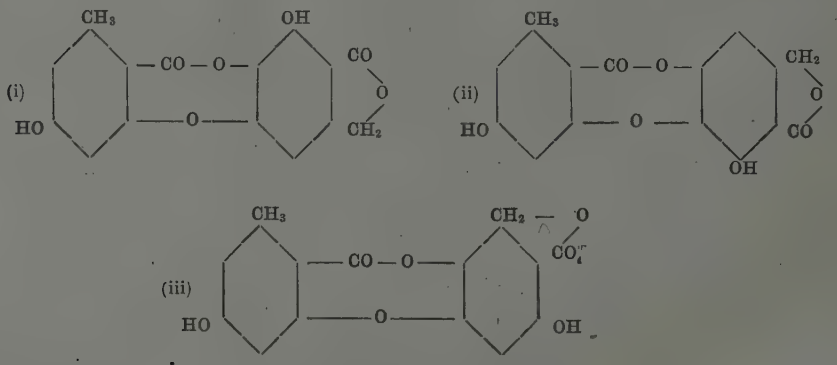
When the product obtained by the action of methyl alcoholic potash on variolaric acid is treated with chlorine, and the products of reaction subsequently reduced, there is isolated from the products 4 : 6 dichlor orsellinic ester. Accordingly we may write the formula for variolaric acid as



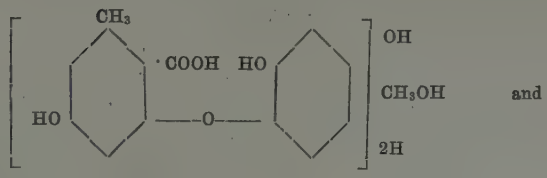
Now the following reactions of variolaric acid and its derivatives are to be noted. Variolaric acid gives a purple reaction with ferric chloride, indicating the presence in the molecule of a carbonyl group ortho to a hydroxyl: neither the acid itself nor the product obtained by opening the lactone bridge with alcoholic potash gives a blood-red reaction with bleaching powder. This latter would indicate that there is no hydroxyl in the position in ring B para to the oxygen bridge. Further it is found that the compound $C_{20}H_{20}O_8$ obtained by the action of methyl alcoholic potash followed by diazomethane on variolaric acid is soluble in methyl alcoholic potash, and that nothing is precipitated on the addition of water, but only on acidification. These results indicate the presence of a lactide group. We may write the formula now



The structure of variolaric acid can then we written in any of the three forms



on the ground that a carbonyl group must be ortho to a hydroxyl group to explain the violet ferric reaction of the original acid. If the α-resorcylic acid obtained in the potash fusion is coming from the B ring of the variolaric acid, then formulae (i) and (iii) are excluded, but while such is probably true, we regard it as unsafe to make this assumption because of the possibility of its arising from oxidation of orcin. However, if we turn to the product obtained by the action of 50 per cent. potash on variolaric acid we find that neither of the two products obtained gives a colour reaction with ferric chloride. For this reason it must be assumed that the carbonyl group that has disappeared in these reactions is that found in ring B, so that the products are

(iv) 

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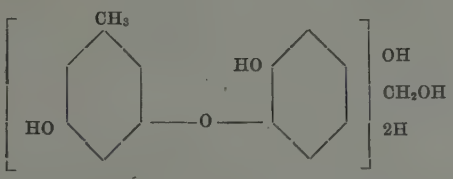
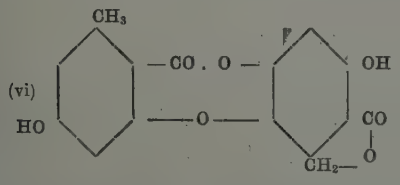
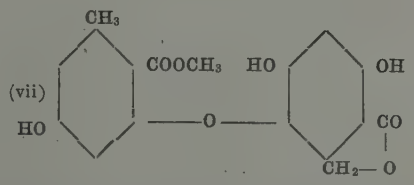
(v) 

Since neither gives a ferric reaction it follows that there is no hydroxyl ortho to the hydroxyl in ring B, so that formula (i) above is excluded.

To differentiate between formulae (ii) and (iii) we have made use of a colour reaction with 2 : 6 dichlor quinone chlorimide in a sodium borate buffer which has been employed by Gibbs (J. Biol. Chem., 1927, **72**, 649) and by Todd (J.C.S., 1940, 1208) to indicate the presence of a hydrogen para to a hydroxyl in an aromatic ring. Davidson (private communication), as a result of his work in these laboratories, has shown that the same colour reaction takes place if there be a COOH group or a chlorine group para to a free hydroxyl in an aromatic ring, but not if the acid group COOH be converted to an ester. By treatment of (iv) with the necessary amount of diazomethane the carboxylic acid group in the A ring was converted into the methyl ester, and the resulting product gave a purple reaction with 2 : 6 dichlor quinone chlorimide, thus showing a free H para to the hydroxyl in the ester of (iv). Consequently formula (iii) for variolaric acid is excluded, and the true constitution is given by the formula (ii) above.

It might be argued that the exclusion of formula (vi)

(vi) 

(vii) 

on the grounds that the product (vii) derived from it by the action of methyl alcoholic potash gives no reaction with bleaching powder is a weak one in view of the possible disturbing influence of the latent CH_2OH in ring B on the bleach reaction. However, the fact that the ester obtained by the action of methyl alcoholic potash on variolaric acid gives a blue coloration with 2 : 6 dichlor quinone chlorimide excludes the formula (vii) for the ester, and is in accordance with formula (ii) for variolaric acid given above. We exclude any arguments based on the positive imide reaction of variolaric acid only, as it is conceivable that the borax buffer, which is alkaline, might cause an opening of the lactone bridge, thus producing a carboxylic acid group para to the

hydroxyl group in ring A, which structure would of course give a positive imide reaction.

Experimental.

Extraction of the Lichen.

The lichen collected at Howth and Portrane, Co. Dublin, at Slishwood, Co. Sligo, and at Dunmore, Co. Clare, was extracted for 20 hours with acetone in a continuous extractor. A light brown crystalline product was deposited in the receiver. This was filtered off, treated twice with small quantities of hot ethyl alcohol, and once with a small amount of hot acetone to remove impurities. It was then crystallized twice from boiling 80 per cent. aqueous acetone yielding fine white crystals of variolaric acid m.p. 296° C. with decomposition and previous browning. Yield of pure material 1 per cent. by weight of lichen.

Variolaric acid is soluble on heating in 80 per cent. aqueous acetone, glacial acetic acid, and with difficulty in alcohol. It gives a purple colour with ferric chloride solution, no colour with bleach solution, and a blue colour with 2 : 6 dichlor quinone chlorimide on standing. With cold concentrated sulphuric acid it gives no colour but turns brown on heating.

Analysis: 4·030 mg. dried in vac. at 105° C. lost 0·270 mg.
(A) 3·760 mg. gave 8·440 mg. CO_2 and 1·140 mg. H_2O.
No Methoxyl found.
(B) 4·061 mg. dried in vac. at room temp. lost 0·326 mg.
3·735 mg. gave 8·390 mg. CO_2 and 1·130 mg. H_2O.
Insoluble in Camphor.

Found: (A) C = 61·2, H = 3·39; H_2O = 6·7. (B) C = 61·3, H = 3·39; H_2O = 8·0.
$C_{16}H_{10}O_7$ requires C = 61·15; H = 3·19.
$C_{16}H_{10}O_7 \cdot 1\frac{1}{4}\ H_2O$ requires H_2O = 6·7;
$C_{16}H_{10}O_7 \cdot 1\frac{1}{2}\ H_2O$ requires H_2O = 8·0.

Action of 10 per cent. aqueous potash on variolaric acid.

0·1 g. of variolaric acid was allowed to stand overnight with 5 c.c. of 10 per cent. aqueous potash, the solution turning brown. On acidification with dilute hydrochloric acid a turbidity formed which on short standing deposited brown crystals. These were filtered off and dissolved in methyl alcohol. The methyl alcohol solution was filtered and water added. On slow evaporation of the methyl alcohol in air brown crystals formed and were filtered off, m.p. 221–3° C. with frothing (on quick heating). The product effloresced in air. This compound gives with ferric chloride a colour between violet and purple, with bleach solution an initial yellow colour which rapidly vanishes, and with 2 : 6 dichlor quinone chlorimide an immediate light blue colour.

Analysis: 3·800 mg. gave 8·040 mg. CO_2 and 1·360 mg. H_2O.
0·683 mg. in 6·447 mg. Camphor. Δ = 13·6° C.

Found: C = 57·7; H = 3·97; M.W. = 300.
$C_{16}H_{12}O_8$ requires C = 57·84; H = 3·62; M.W. = 332.

Action of 50 per cent. aqueous potash on variolaric acid.

1 g. of variolaric acid was heated to gentle boiling for 5 hours in 10 c.c. of 50 per cent. aqueous potash, a hydrogen atmosphere being maintained all the time. It was cooled, poured on ice, acidified with dilute sulphuric acid, and allowed to stand overnight. It was then filtered, the filtrate extracted with ether, and the ether solution extracted with 3 per cent. sodium bicarbonate solution.

Sodium-bicarbonate-insoluble fraction.—The ether was dried over sodium sulphate and evaporated, leaving a clear colourless gum. This was taken up in a small quantity of acetic acid, and 3 volumes of benzene added to the hot solution. On standing small white crystals were deposited, m.p. 194–5° C.

This compound gives no colour with ferric chloride solution, with bleach solution a brown colour, with 2 : 6 dichlor quinone chlorimide an immediate purple colour, and with cold concentrated sulphuric acid a yellow colour changing to greenish yellow on heating. It contains no acetyl. The material may also be crystallised from warm water.

ANALYSIS: Compound dried in vacuo at 100° C.—No loss.
4·107 mg. gave 9·580 mg. CO_2 and 2·040 mg. H_2O.
0·546 mg. in 5·273 mg. camphor. $\triangle = 14·5°$ C. (Slight decomposition.)

Found: C = 63·60; H = 5·53; M.W. = 264.
$C_{14}H_{14}O_5$ requires C = 64·1; H = 5·34; M.W. = 262.

Sodium-bicarbonate-soluble fraction.—The sodium bicarbonate solution was acidified, and gave a water-soluble oil, which was extracted with ether. The ethereal solution was dried over sodium sulphate and evaporated, leaving a reddish brown oil. This was dissolved in a little hot acetic acid, and an equal volume of benzene added to the hot solution. On short stand very small white crystals were deposited. The melting point depends on the rate of heating; slow heating 188·5° C. (frothing); rapid heating 194–6° C. (frothing).

This compound gives a brown colour with ferric chloride solution, a greenish yellow colour with bleach solution, and with 2 : 6 dichlor quinone chlorimide a light purple colour. It contains no acetyl.

A mixture with the bicarbonate-insoluble fraction melts at 183·5° C.

ANALYSIS: (A) 3·893 mg. gave 8·430 mg. CO_2 and 1·670 mg. H_2O.
(B) 3·902 mg. gave 8·360 mg. CO_2 and 1·670 mg. H_2O.
0·421 mg. in 4·012 mg. camphor gave $\triangle = 14·0°$ C.

Found: (A) C = 59·1; H = 4·77. (B) C = 58·43; H = 4·76.
M.W. = 277.
$C_{15}H_{14}O_7$ requires C = 58·83; H = 4·58; M.W. = 306.

Methylation of the bicarbonate-insoluble compound, m.p. 194–5° C.—(*a*) With methyl sulphate; 0·1 g. of sodium-bicarbonate-insoluble compound was dissolved in 5 c.c. of 3·5 normal caustic soda. 1·5 c.c. of methyl sulphate was added, and the mixture shaken vigorously. On standing a precipitate formed, which was filtered, washed with water, and dried in vacuo. This on recrystallisation from one volume of benzene and four volumes of 60/80° petrol ether deposited clusters of thick prisms, m.p. 128–9° C.

This compound develops a faint pink colour on standing with 2 : 6 dichlor quinone chlorimide.

Analysis: 3·940 mg. gave 9·620 CO_2 and 2·240 mg. H_2O.
4·401 mg. gave 7·120 mg. AgI.
Found: C = 66·6; H = 6·32; OCH_3 = 21·3.
$C_{14}H_{12}O_3 \cdot (OCH_3)_2$ requires C = 66·2; H = 6·21; OCH_3 = 21·4.

The same compound was obtained when the methylation was carried out in boiling caustic soda solution.

(*b*) *With diazomethanes.*—0·2 g. of the bicarbonate insoluble compound was dissolved in 10 c.c. of dry acetone. Excess diazomethane was added (2 g. nitroso methyl urea), and allowed to stand corked for one day, when a slight precipitate formed. On evaporation of the solvents an oil was got which we did not succeed in crystallising.

Methylation of the bicarbonate-soluble compound, m.p. 194–6° C.—(*a*) Using excess diazomethane; 0·4 g. of the bicarbonate-soluble compound in 20 c.c. of dry acetone was treated with excess diazomethane (5 c.c. nitroso-N-methyl urethane), and allowed to stand overnight. The solvents were evaporated in air, and the residue crystallised from a mixture of benzene and 60/80° petrol ether, when clusters of pointed prisms were deposited, m.p. 108–9° C.

Analysis: 3·830 mg. gave 8·750 mg. CO_2 and 2·100 mg. H_2O.
5·121 mg. gave 12·500 mg. AgI.
Found: C = 62·3; H = 6·11; OCH_3 = 32·2.
$C_{15}H_{10}O_3 \cdot (OCH_3)_4$ requires C = 62·98; H = 6·08; OCH_3 = 34·26.

(*b*) Using one molecular proportion of diazomethane, 1·5 g. of the bicarbonate-soluble compound in dry acetone was treated with sufficient diazomethane to methylate only the carboxyl group. On evaporating the solvents an oil was got which was taken up in ether, the ethereal solution extracted with a small quantity of 3 per cent. sodium bicarbonate solution, dried over sodium sulphate, and evaporated, leaving a white solid which on crystallisation from acetic acid and 60/80° petrol ether gave rhombic crystals, m.p. 217–8° C.

This compound develops a purple colour with 2 : 6 dichlor quinone chlorimide.

Analysis: No loss when dried in vacuo at 100° C.
4·051 mg. gave 8·880 mg. CO_2 and 1·780 mg. H_2O.
4·442 mg. gave 3·150 mg. AgI.
Found: C = 59·78; H = 4·88; OCH_3 = 9·40.
$C_{15}H_{13}O_6 \cdot (OCH_3)$ requires C = 60·0; H = 5·0; OCH_3 = 9·70.

Attempts to acetylate the bicarbonate-soluble and insoluble products referred to above gave oily products which could not be obtained crystalline.

Diacetate of variolaric acid.

0·05 g. of variolaric acid was treated with 5 c.c. of acetic anhydride and 3 drops of sulphuric acid, in which it dissolved. It was allowed to stand for two hours, and was then poured into 15 c.c. water. A white precipitate formed, which was filtered off, dried, and recrystallised from 40 c.c. of methyl alcohol. On standing small rhombs were deposited, m.p. 245–6° C. with previous colouring.

ANALYSIS: 4·108 mg. gave 9·080 mg. CO_2 and 1·340 mg. H_2O.
8·340 mg. required 4·34 c.c. $N/100$ NaOH.

Found: C = 60·29; H = 3·65; CH_3CO = 22·4.
$C_{16}H_8O_7 \cdot 2CH_3CO$ requires C = 60·3; H = 3·52; CH_3CO = 21·6.

Action of Diazomethane on variolaric acid.

0·11 g. of variolaric acid was suspended in 10 c.c. of acetone, treated with excess diazomethane (3 c.c. nitroso-N-methyl urethane), and allowed to stand for two days. The solution filled with a white flocculent solid which did not dissolve. It was then poured into a dish, and the solvents evaporated. A white residue was obtained, which was recrystallized from a mixture of equal volumes of benzene and methyl alcohol. White hairlike crystals were deposited, m.p. 260–1° C. with blackening.

ANALYSIS: 4·057 mg. gave 9·430 mg. CO_2 and 1·570 mg. H_2O.
4·523 mg. gave 6·000 mg. AgI.

Found: C = 63·37; H = 4·33; OCH_3 = 17·5.
$C_{16}H_8O_5 \cdot (OCH_3)_2$ requires C = 63·1; H = 4·1; OCH_3 = 18·1.

Action of 50 per cent. aqueous potash on variolaric acid dimethyl ether.

1·0 g. of variolaric acid dimethyl ether was heated in 10 c.c. of 50 per cent. aqueous potash in a hydrogen atmosphere for 5 hours. The mixture was cooled, poured on ice, and acidified, when a white precipitate formed which was practically insoluble in ether. This was recrystallised from aqueous methyl alcohol, and yielded small white crystals, m.p. 246° C. (gas evolution).

This compound gives no colour reaction with ferric chloride solution, bleach solution, or 2 : 6 dichlor quinone chlorimide.

ANALYSIS: No loss when dried in vac. at 100° C.
3·660 mg. = 8·050 mg. CO_2 and 1·500 mg. H_2O.
3·152 mg. = 3·750 mg. AgI.

Found: C = 59·95; H = 4·55; OCH_3 = 15·7.
$C_{16}H_{10}O_6 \cdot (OCH_3)_2$ requires C = 60·00; H = 4·44; OCH_3 = 17·2.

The same compound was obtained by the action of boiling 10 per cent. aqueous potash on variolaric acid dimethyl ether. The product, crystallised from methyl alcohol, had m.p. 248–9° C. not depressed on admixture with the product obtained in the previous experiment.

ANALYSIS: 4·275 mg. gave 5·580 mg. AgI.

Found: $OCH_3 = 17·25$.

$C_{16}H_{10}O_6·(OCH_3)_2$ requires $OCH_3 = 17·2$.

Action of 5 per cent. methyl alcoholic potash on variolaric acid.

0·2 g. of variolaric acid was allowed to stand for 24 hours in 10 c.c. of 5 per cent. methyl alcoholic potash in the cold, when complete solution took place. It was poured into dilute hydrochloric acid, and let stand overnight to allow the alcohol evaporate in air, when a precipitate formed. This was filtered, washed with water, dried, and recrystallised from methyl alcohol and water, which yielded minute clusters of white crystals, m.p. 243° C. with decomposition.

This compound gives a violet colour with ethyl alcoholic ferric chloride solution, and with 2 : 6 dichlor quinone chlorimide a blue colour which immediately changes to dark grey-blue. When the compound is dissolved in ethyl alcohol, and bleach solution added drop by drop it gives at first a yellow colour, then brown, and on continued addition a reddish-purple colour.

ANALYSIS: 3·902 mg. dried in vac. at 100° C. lost 0·261 mg.
3·641 mg. gave 7·860 mg. CO_2 and 1·380 mg. H_2O.
4·578 mg. gave 3·110 mg. AgI.
0·392 mg. in 4·906 mg. camphor. $\triangle = 8·2°$ C.

Found: C = 58·87; H = 4·21; $OCH_3 = 8·98$; $H_2O = 6·7$; M.W. = 360.

$C_{16}H_{11}O_7·(OCH_3)$ requires C = 58·95; H = 4·05; $OCH_3 = 8·96$; M.W. = 346.

$C_{16}H_{11}O_7·(OCH_3)·1\frac{1}{2}H_2O$ requires $H_2O = 7·2$.

Action of diazomethane on ester, m.p. 243° C.

0·18 g. of the previous compound was dissolved in 10 c.c. of acetone, and allowed to stand corked with excess diazomethane (3 c.c. nitroso-N-methyl urethane) overnight. The solvents were evaporated in air, the residue dried in vacuum, and recrystallised from methyl alcohol, yielding long white needles, m.p. 181–2° C. The compound gives no colour reaction with ferric chloride solution. It is soluble in methyl alcoholic potash, the solution on dilution with water and removal of the methyl alcohol giving no precipitate until acidified.

ANALYSIS: 4·536 mg. gave 10·270 mg. CO_2 and 2·110 mg. H_2O.
4·230 mg. gave 10·160 mg. AgI.

Found: C = 61·73; H = 5·17; $OCH_3 = 31·73$.

$C_{16}H_8O_4·(OCH_3)_4$ requires C = 61·85; H = 5·17; $OCH_3 = 31·96$.

When this experiment was repeated by letting the compound stand for 3 days with excess diazomethane the same product was obtained. M.p. 182° C., unchanged when mixed with the above compound. The same product was also obtained when the methylation was carried out with potassium carbonate and dimethyl sulphate in boiling acetone.

Potash fusion of variolaric acid.

20 g. of caustic potash pellets and 2 c.c. of water were heated to 100° C., and then 1 g. of variolaric acid was added slowly with stirring. The temperature of the melt was raised to 250° C., and maintained at that temperature for 20 minutes and then at 310° C. for 5 minutes, when the colour of the melt changed to red. The melt was cooled, dissolved in water, carbonated for 30 minutes, and then acidified with dilute sulphuric acid. The acidified solution was extracted with ether, and the ethereal solution extracted, first with 3 per cent. sodium bicarbonate, and then with 3 per cent. sodium hydroxide, and the solutions worked up as follows:—

(*a*) The bicarbonate solution was acidified with dilute sulphuric acid, extracted with ether, the ethereal solution dried with sodium sulphate, and evaporated, leaving a brown gum containing some crystals. This was boiled with benzene, and gave a dark soluble residue, which was dissolved in water, cleared with charcoal, filtered, and the aqueous solution evaporated to dryness. A white residue was obtained, which was dissolved in acetic acid, filtered, and two volumes of benzene added to the filtrate, when white plates were obtained, m.p. 230–232° C. not depressed on admixture with *a*-resorcylic acid.

ANALYSIS: 2·958 mg. vac dried at room temperature—no loss.
2·958 mg. gave 5·880 mg. CO_2 and 1·070 mg. H_2O.

Found: C = 54·2; H = 4·06;
$C_7H_6O_4$ requires C = 54·5; H = 3·9.

(*b*) The sodium hydroxide solution was acidified with dilute sulphuric acid, extracted with ether, the ethereal solution dried over sodium sulphate and evaporated, leaving a brown oil. This was dissolved in hot chloroform, and some drops of hot water added. On standing large platelike crystals were precipitated, m.p. 57–58° C. unchanged when mixed with orcin. This compound gave no colour with ferric chloride solution, and a blood-red colour with bleach solution.

Action of chlorine followed by reduction on the product m.p. 243° C. obtained by the action of methyl alcoholic potash on variolaric acid.

1 g. of this compound was suspended in 75 c.c. of chloroform, and treated with 41 c.c. of carbon tetrachloride containing 2 g. chlorine, and left stand corked 5 days. The chlorine and hydrochloric acid were then removed by bubbling dry air through the solution. The solvents were evaporated, leaving a greenish brown oil. This oil was reduced by dissolving it in 30 c.c. of glacial

acetic acid and adding gradually 3 g. of stannous chloride in 60 c.c. of 20 per cent. hydrochloric acid. The colour changed to reddish-brown, and a gum separated on the walls of the flask. The material was diluted with water, extracted with chloroform, and the chloroform evaporated. The residue was taken up in ether and extracted with 3 per cent. sodium bicarbonate. The ethereal solution was evaporated to dryness, and the residue distilled in superheated steam, when crystals passed over. These were collected, crystallised twice from ligroin, and once from aqueous methyl alcohol, yielding fine needles, m.p. 115° C. (corr.) not depressed when mixed with 2 : 4 dichlor o-orsellinic acid methyl ester (Nolan and Murphy, Sci. Proc. R.D.S., **22**, 317).

This compound gives a purple colour with ferric chloride and a blood-red colour with bleach solution.

2 : 6 dichlor-para-orsellinic acid methyl ester.

0·34 g. 3 : 5 dihydroxy p-toluic acid methyl ester was dissolved in 5 c.c. of chloroform, and treated with 7·1 c.c. carbon tetrachloride containing 0·33 g. chlorine, the whole being cooled. After standing corked for 1½ hours excess chlorine was removed by bubbling dry air through the solution. The solvents were evaporated in air, and the residue crystallised from methyl alcohol or benzene, yielding small white crystals m.p. 167–9° C.

This compound gives a dark blue colour with ferric chloride solution, no colour with bleach solution, and with 2 : 6 dichlor quinone chloromide a pink colour. The material was dried in vacuum at 80° C. before analysis.

Analysis: 3·282 mg. gave 5·220 mg. CO_2 and 0·970 mg. H_2O.
5·959 mg. gave 6·780 mg. AgCl.
4·980 mg. gave 4·810 mg. AgI.

Found: C = 43·38; H = 3·3; Cl = 28·14; OCH_3 = 12·7.
$C_7H_5O_2Cl_2(COOCH_3)$ requires C = 43·03; H = 3·2; Cl = 28·28; OCH_3 = 12·3.

Alcohol extraction of Lichen.

The air dried lichen from the acetone extraction was boiled for three hours with ethyl alcohol. On filtering and evaporating the solvent a small quantity of green extract was obtained, which was dried, extracted with 50 c.c. of water, and the water evaporated, leaving a brown gum. This was washed with 35 c.c. of warm methyl alcohol. The portion insoluble in methyl alcohol was left as a brown powder, which on crystallisation from boiling methyl alcohol yielded white crystals, m.p. 162–3° C. not depressed on admixture with mannitol.

One of us (D. M.) acknowledges with thanks a grant from the Department of Industry and Commerce which has enabled him to collaborate in this investigation.

No. 9.

SALMON AND SEA TROUT OF THE WATERVILLE (CURRANE) RIVER.

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AND

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[Read 15 December, 1942. Published separately 7 April, 1943.]

The Waterville River and its Tributaries.

The Waterville River and the Sligo River are the only rivers in Éire to open for salmon fishing by means of nets and weirs on New Year's Day, the number of fish taken at Waterville in the month of January often exceeding the catch of any other month of the fishing season. The main fishery on the river, known as the Waterville Fishery, is held under an ancient charter, and consists of an old-established weir and a pool which is fished by means of a draft net. Under the provisions of an Act entitled "An Act to amend the laws relating to Fisheries in Ireland" (26 and 27 Vict. Cap. 114) all fishing weirs had to be provided with a free gap for the passage of fish, constructed in accordance with certain well-defined rules. In the case of chartered or patent weirs existing in 1863 on rivers less than forty feet wide a concession was made, and the authorities were, by Section 11 of the said Act, empowered, if they thought fit, to extend the normal weekly close time by twenty-four hours in lieu of a free gap. The provisions of this section have been availed of in the case of the Waterville Salmon Weir, and a weekly close time from 12 o'clock noon on Friday to 12 o'clock noon on the Monday following operates instead of the usual weekly close time from 6 a.m. on Saturday to 6 a.m. on the Monday following. Netting in the pool below the weir is also restricted by statute and by-law to the same period. It is also interesting to note that after their capture both salmon and sea trout are often kept alive in a fish box for several days before despatch to the London and other markets.

The Waterville River is some 500 yards long and enters the sea through a cutting in a boulder storm beach. It drains Lough Currane, a sheet of water about 3½ miles long, 2 miles wide, and up to 90 feet deep in the deepest parts. The character of the river below the Salmon Weir has changed somewhat in recent years, because at one time this portion of the river was looked upon as tidal, whereas at present it is neither salt nor is the height of water in the river influenced by the majority of tides. It is obvious, however,

that the prevailing heavy seas and their effect on the contour of the beach may have been responsible for this change.

There are numerous islands in Lough Currane, which forms one of the most attractive features of Waterville as a fishing and holiday resort. At the eastern end of the lake there are two important tributaries, of which the northern, the Cummeragh River about four miles long is the larger, and is fed by a number of lakes (Loughs Derriana, Cloonaghlin, Namona being the chief) and mountain streams, to which salmon and sea trout resort in large numbers to spawn. The mouth of the river is in a bay, of which the entrance is partly closed by small islands. A considerable number of salmon and sea trout are taken by angling in the lower stretches from about March onwards. The upper

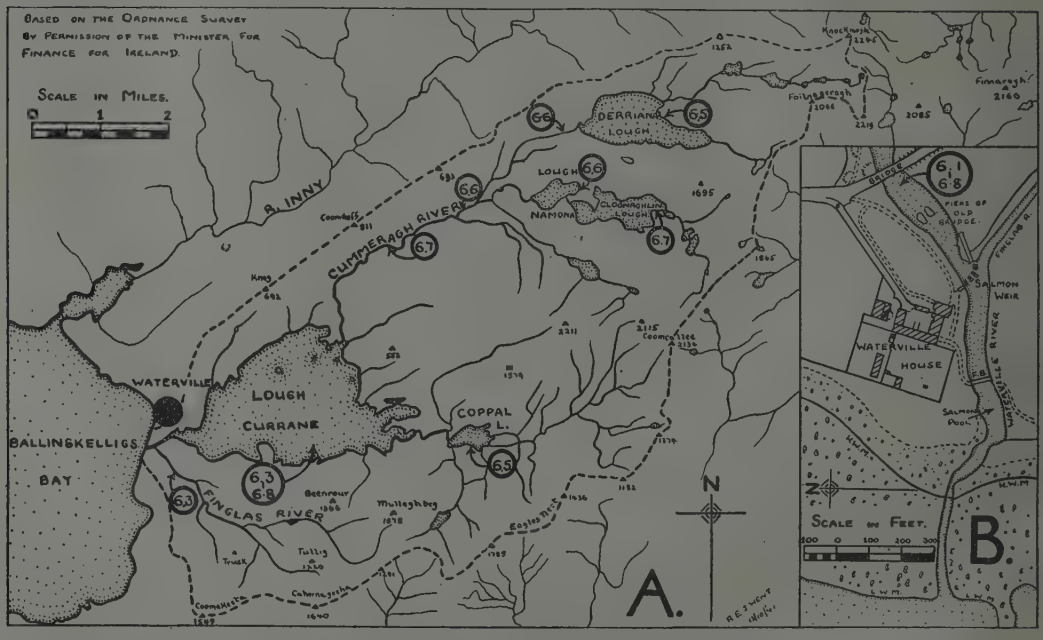

Fig. 1.—A. Sketch map of the catchment area of the Waterville River and its tributaries.

B. Sketch map of the Waterville Fishery.

(Figures inside circles indicate pH values in normal heights of water.)

reaches of tributaries of the Cummeragh River are little more than mountain torrents, especially those draining part of the southern slopes of Knockmoyle (2,245 feet high) and adjacent mountains. The other important tributary, entering Lough Currane to the south of the mouth of the Cummeragh River, is a stream about a mile long which comes down from Coppal or Isknagakiny Lough, and is frequented by a fair number of salmon and sea trout. Another tributary, the Finglas River, entering the Waterville River just below the

Salmon Weir occasionally holds salmon and sea trout during the season, the main run being of almost ripe fish after the close of the fishing season.

As the season progresses salmon entering Lough Currane from the sea tend to assemble near the mouths of the tributary rivers, awaiting favourable conditions which arise when a flood comes down after heavy rain. In consequence of this, netting in the lake has been prohibited by by-law in order to maintain the stocks of fish.

The whole of the waters of the Waterville River and its tributaries flow over rocks belonging to the Old Red Sandstone, which are overlaid in places by glacial drift. As the rocks of the river basin contain very little lime the waters of the various tributaries and lakes are either slightly acid or neutral. In Fig. 1A the pH values of the waters of selected localities have been indicated, and in Table I (see Appendix for tables) typical analyses of the waters from certain localities in the catchment area have been given. The total area of the basin of the Waterville River amounts to 46 square miles. In Fig. 1A the disposition of the Waterville River, its lakes and tributaries has been indicated.

Material.

The material used in this investigation consists of 698 satisfactory sets of scales and measurements of salmon, and 433 similar sets from sea trout captured in both the weir and net fishery on the Waterville River (see Fig. 1B). The bulk of the material was collected during the year 1941 under the guidance of one of us (T. S. B.), but a number of sets relating to both species of fish were collected in previous years by Mr. James W. Butler, the owner of the Waterville Fishery. The number of fish sampled in each month is given in the following table:—

Month.	Salmon.	Sea Trout.
January	147	
February	129	51
March	133	
April	53	163
May	62	115
June	114	96
July	60	8
TOTAL	698	433

Examination of the above table, and of confidential returns of the catch of 1941, indicates that the numbers of fish sampled were fairly representative of the total catches, save in that the runs of sea trout after the 15th July were not sampled. There were unavoidable fluctuations in the percentage of fish sampled, but by suitable arithmetical manipulation the data supplied can be used to obtain a fairly accurate idea of the incoming salmon and spring sea

trout populations. Wherever possible the weighted figures have been given in the tables.

Salmon begin to run into the Waterville River in fairly large numbers during the month of December, and continue to run in force until the beginning of August. The legal season for netting in the Waterville River is from 1st January until 15th July, both days inclusive, and it is obvious that the material will not contain samples of the runs of salmon in December or those after 15th July. As will be shown later, two age groups, namely the 1 + and 2 winters age groups, formed nearly 90 per cent. of the total catch of salmon, and in consequence the results will not be materially affected by the lack of samples from December and after the 15th July. In the case of sea trout, which normally begin to run in February or March, the absence of samples after 15th July means that the later running sea trout will be absent, so that the material is not representative of the runs of the sea trout as a whole, but may be considered a fairly adequate sample of the sea trout runs up to the end of the netting season, i.e. of the spring runs.

All fish were measured from tip of the snout to the fork of the tail, and the length was recorded to the nearest tenth of an inch.

Age and Growth of the Salmon.

Smolt Ages.

The distribution of the various smolt ages in each age group is given in Table 3. The bulk of the fish migrated as two-year smolts, and the proportion of three-year smolts exceeded that of the one-year smolts. The Waterville River resembles in this respect the Rivers Owenduff and Erne (Went, 1941 and 1942), but differs considerably from the River Shannon and Ballisodare River (Went, 1938, 1940 and 1941). The percentage of young smolts was slightly greater in the small spring fish than in the grilse.

Age Groups.

The fish whose scales were satisfactory for age determinations were divided into four groups of maiden or unspawned fish and one group of previously spawned fish usually marked "with S.Ms." (with spawning marks on the scales). The results have been given in Table 4. Grilse (1 + winters) and small spring fish (2 winters) formed together 88·3 per cent. of the total catch. Small spring fish were most abundant, with a percentage of 55·4 per cent. It is surprising that, in a river having such early runs of salmon as the Waterville River, the proportion of large spring fish is so small. Apart from the scale readings, the fact that the average weight of fish in the first four months of the year did not rise above 12·0 lb. in 1941 confirms the view that large spring fish were not numerous (Table 2). A few summer fish were taken in April, in May they formed about one-third of the total catch, and in June and July they formed over 90 per cent. of the whole catch (Table 4). Grilse were most plentiful in July, and if samples had been taken during the whole of that month the figure would have been somewhat in excess of that given in Table 5. By the close of

the netting season the runs of grilse had, however, diminished in size very considerably. The peak of the small summer fish (2 + winters) run was in June, whilst most previously spawned fish, the bulk of which had spawned for the first time as grilse, were taken in July. The best month for small spring fish (2 winters) was February. In the months of April and May the runs of fish were relatively poor.

It might be asked whether the results for 1941 can be applied to previous years. Consideration of the confidential returns of the Waterville Fishery over some ten years revealed that, in general, the runs in 1941 showed a similar distribution in time to the runs in previous years. The year 1941 was probably not abnormal in most respects, and similar conditions would appear to have prevailed over a number of years.

The previously spawned fish formed 7·2 per cent. of the total catch. One fish, out of the 44 of this group examined, had two spawning marks on its scales, whilst the remainder had one spawning mark only. Previously spawned fish can be classified on (1) "Absence," the time spent feeding in the sea between what would have been, but for the intervention of man, two successive spawnings, and (2) the age at first spawning. In Table 6 the previously spawned fish have been so classified. As is usual, the spring fish adopted the long absence habit, whilst the grilse adopted the short or very long absence habit.

Divided Migration and Return.

Table 7 gives the years in which the fish of the 1941 catches were hatched. It will be seen that in 1941 the year classes of 1938 (fish in their fourth year) and 1937 (fish in their fifth year) were the most important. From the commercial point of view, however, the 1937 year class was considerably more important than is indicated by its numerical proportion in the total catch owing to the comparatively high average weight of its members.

Size Distribution.

Table 8 gives the estimated percentage of fish occurring in each two-inch class interval. Fish having lengths between 23·95 and 33·95 inches formed 85 per cent. of the total catch. The class interval of 30 (fish having lengths between 29·95 and 31·95 inches) formed about one-quarter of the total catch in 1941.

Condition Co-efficient.

When considering the relationship between the weight and length of salmon it is usual with workers using British units of measurements and weight to adopt a formula devised by Menzies (1921) when he was investigating salmon of the Scottish Dee. The relationship, called the condition co-efficient (K), is determined from the following formula:—$K = W/(L^3 \times 0{\cdot}00036)$, where W = weight in lb. and L = length in inches. This formula gave a value approximating to unity for salmon of the Scottish Dee, which were therefore taken as a standard. Unfortunately salmon of the Scottish Dee are somewhat lighter, length for length, than fish from most of the rivers investigated to date.

It might be mentioned that if metric units are used, and a condition factor is calculated using the formula, 100,000 W/L^3, where W = weight in kilogrammes and L = length in centimetres, the results closely approximate to those given by Menzies' formula (they actually differ by 0·4 per cent. only). Despite the close approximation of the results it would seem desirable that a standard method should be adopted for calculating the relationship between weight and length in salmon and other salmonidae. The metric formula, which, unlike Menzies' formula, does not imply the idea of a standard fish, would appear to be the most convenient one for general adoption.

Some criticism might be levelled at a worker for using British instead of Metric units in scientific work. With a valuable fish such as the salmon it is obviously impossible to obtain large numbers of fish for investigation in the laboratory, and recourse has to be had to other methods of obtaining material. Usually this is done, as in the present case, by arranging for some reliable person to make collections of sets of scales and measurements, etc., of salmon taken in a fishery on the river which it is desired to investigate. It has been one of the author's experiences in Ireland that the use of metric units in the collection of such data leads to inaccuracies and obvious mistakes, and on account of this all measurements have been made with what were to the collectors the familiar British units of weight and length.

Owing to difficulties which arose from the arrangements for the despatch of the fish to the markets it was not found possible to weigh all the fish. Selections of fish were, however, weighed during each month, and the condition co-efficients have been calculated for each age group from these data. The mean condition co-efficient on Menzies' scale for each age group is given in Table 9, the mean value for all fish being 1·08. Spring fish were in better condition than the summer fish, the average condition co-efficients being 1·10 and 1·05 respectively. In this respect salmon of the Waterville River resemble those of the River Shannon (Went, 1938 and 1940). The mean condition co-efficients were, however, considerably lower than those of salmon from Irish rivers already investigated (Southern, 1928; Went, 1938, 1940, 1941, and 1942).

Average Sizes.

Details of the average lengths and weights will be found in Table 10. As only a proportion of the fish was weighed, the average weight of each age group was calculated from the average length and the average condition co-efficient. That these estimated average weights were fairly accurate is shown by the fact that the estimated average weight of all fish examined was 10·06 lb., whereas the actual average weight per fish as indicated by the confidential returns was 9·9 lb. (Table 2).

(*a*) Grilse (1 + winters). Number examined = 157.

	inches.	lb.
Minimum	22·1	3·9 (actual)
Average	25·4	5·9 (estimated)
Maximum	30·0	—

The first grilse was taken in 1941 on 29th May. The minimum length was recorded on two occasions, namely, 5th and 7th June, whilst the maximum length was recorded on 26th June.

(*b*) Small spring fish (2 winters). Number examined = 464.

	inches.	lb.
Minimum	27·0	—
Average	30·9	11·7 (estimated)
Maximum	37·5	—

The minimum length was recorded on 14th February, whilst the maximum length was recorded on 23rd May.

(*c*) Small summer fish (2 + winters). Number examined = 20.

	inches.	lb.
Minimum	26·0	5·5 (actual)
Average	31·8	11·6 (estimated)
Maximum	36·0	—

The shortest fish was captured on 13th June, and the maximum length was recorded on two occasions, namely, 23rd May and 20th June.

(*d*) Large spring fish (3 winters). Number examined = 13.

	inches.	lb.
Minimum	35·0	16·0 (actual)
Average	38·1	22·7 (estimated)
Maximum	42·5	30·0 (actual)

The minimum length was recorded on two occasions, namely, 24th January and 25th April, whilst the maximum length was recorded on 24th January.

(*e*) Previously spawned fish (with S.Ms.). Number examined = 44.

	inches.	lb.
Minimum	26·0	—
Average	34·5	16·7 (estimated)
Maximum	41·5	25·5 (actual)

The smallest fish was captured on 26th June and had adopted the short absence habit, having spawned for the first time as a grilse. The largest fish, which had adopted the long absence habit, was captured on 14th March, having spawned for the first time as a small spring fish. Average weights in this group are of little value, because it consists of a heterogeneous collection of fish having only one common character, namely, the presence of spawning marks on their scales.

Erosion.

Erosion, or more properly absorption, of the substance of the scales begins to take place when the gonads of salmon are ripening. Usually this takes place in fresh water, but it can also take place in the sea if the fish has been delayed

in reaching fresh water for any reason. In the material at the authors' disposal six spring fish showed slight erosion, none of which, however, exceeded 3° on Menzies' scale.

Calculated Lengths.

The length of each "maiden" or unspawned fish at the end of each year of its life was calculated in the usual way, from measurements of the scales, i.e. on the assumption that the growth of the scale is strictly proportional to the growth of the fish.

A. River Life.

The relationships of the parr lengths are dealt with in this section. In Table 11 the calculated lengths of the parr at the end of each winter of freshwater life in the various smolt classes are given (see also Fig. 2A). It will be seen that the most rapidly growing smolts migrated first. At the end of the first year in fresh water the one-year smolt class was, on the average, longer than the two and three-year smolt classes, the two-year smolt class being superior in length to the three-year smolt class. Similar conditions have also been described for other rivers in Ireland (Went, 1938, 1940, 1941, and 1942).

In previous publications dealing with the salmon of the Rivers Shannon, Ballisodare, Owenduff, and Erne, it was stated that smolts in these rivers fell naturally into two groups, namely those which exhibited freshwater growth in the spring prior to migration to the sea, and those which showed no growth in the spring prior to the smolt migration. In the Waterville River a similar condition was observed. If new freshwater growth is limited to one or two annuli it is often difficult to define the limits between the winter and spring growth. To eliminate this difficulty the smolts were divided into two groups, namely:—

(*a*) One group, termed type A smolts, which showed little or no new growth in fresh water in the spring prior to migration as a smolt, i.e. new growth restricted to two new annuli or less, and

(*b*) one group, termed type B smolts, containing those fish which have made considerable growth in fresh water in the spring prior to migration to the sea.

The distributions of the two smolt types in the various smolt classes are given in Table 12 as percentages of the total maiden fish. Almost three-quarters of the total catch could be referred to the two-year type B smolt class, and in this respect salmon of this river resemble those of the Rivers Owenduff and Erne (Went, 1941 and 1942).

The average lengths of fish belonging to the two smolt types in the various smolt classes have been calculated, and the results are recorded in Table 13. At the end of each year of freshwater life the type A smolts were longer on the average than type B smolts of the same smolt class, Fig. 2B. Similar conditions have already been described for salmon from the Rivers Shannon,

Ballisodare, Owenduff, and Erne, and a hypothesis was advanced to explain this phenomenon. Briefly the hypothesis is that salmon parr must attain some physiological condition associated, at least as an index, with a minimum size before the smolt migration takes place. Jones (1939), dealing with the salmon of the Cheshire Dee, raises objections to the acceptance of this view on the

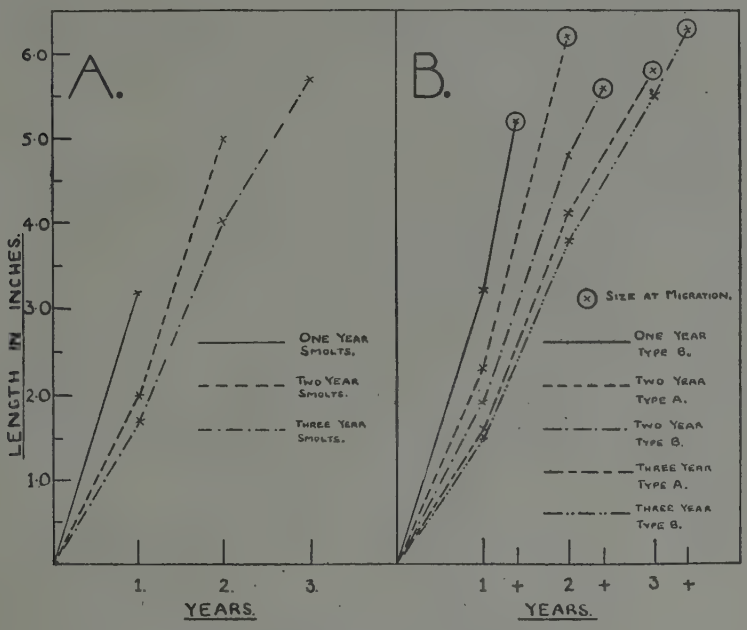

FIG. 2.—Fresh water growth in salmon.

A. Growth curves of the three smolt classes.

B. Growth curves of the different growth types.

grounds that he finds a big variation in the calculated lengths of the smolts from that river, and that some very large smolts have been observed. This criticism is based, to some extent, on a misunderstanding of this hypothesis which does not suggest that migration occurs immediately an individual reaches a certain length. Dealing as we are with biological material, it is not to be expected that the separation of migrating salmon parr would occur with mathematical precision such as is usually the case in forms of mechanical separation. If the validity of the hypothesis is assumed, it might be expected that lengths of the individual smolts, if plotted, would produce a normal curve with the mean corresponding with the value of the "minimum size." In this way the variation observed by Jones is explained to some extent.

In previous publications the calculated smolt lengths of salmon from the Rivers Shannon, Ballisodare, Owenduff, and Erne, have been given and are recapitulated in the following table for sake of convenience:—

Shannon, 1928 (Went, 1940)	5·54 inches
Ballisodare (Went, 1940)	4·87 „
Owenduff (Went, 1941)	4·9 „
Erne (Went, 1942)	5·6 „
Waterville	5·7 „

It will be seen that there is a considerable variation in the average smolt length in the five Irish rivers investigated. If a variation occurs in different rivers there is no reason to believe that similar variations do not occur in the different portions of a river, particularly in a large river where different tributaries run over rocks of different geological formations. This would also explain to some extent the facts observed by Jones in the Cheshire Dee.

In general no difficulty is experienced in distinguishing between sea and freshwater growth as exhibited by the scales, and therefore it is quite possible to calculate the length of each individual smolt. The average lengths of the smolts in each smolt class are given in Table 11. It will be seen that the average size rose as the smolt age increased. With an average length of 5·7 inches, smolts of the Waterville River are similar in size to those of the Rivers Shannon and Erne, but considerably larger than those of Ballisodare and Owenduff Rivers. From examination of Table 14 it would appear that no differentiation in length in fresh water occurs in the various age groups. The average smolt lengths in the two-year smolt class show little variation in the various age groups.

B. Sea Life.

The length at the end of every year in the sea was calculated for every fish with suitable scales. In Table 14 these lengths are given for the various age groups. At the end of the first sea winter the grilse have a greater average length than any other age group; this being more readily seen by comparing all grilse with the remaining age groups as follows:—

Age Group.	No. examined.	Average length at end of 1st sea winter.
Grilse	156	20·2 inches
2, 2 +, and 3 winters age groups combined ...	486	19·3 „

C. Correlation Tables.

In this section it is proposed to discuss the relationship between the growth made in the various years of life.

(1) Correlation between parr lengths at end of the first and second winters in the two-year smolt class.

Table 15 gives the correlation between the length of parr at end of the first and second winters in the two-year smolt class. It will be seen that if the fish are grouped in ascending order according to the size at the end of the first winter the increments made in the second year show a gradual increase.

(2) Correlation between smolt length and length at the end of first sea winter.

It has already been mentioned that the grilse were, on the average, longer at the end of the first sea winter than any other group. In the following discussion the grilse and small spring fish have been considered separately and Table 16 gives the correlation between smolt length and length at the end of the first sea winter in each of these age groups. The smolts which were longer than average maintained their superior length at the end of the first sea winter.

(3) Correlation between lengths at the end of first and second sea winters in small spring fish.

Table 17 gives the correlation between the lengths at the end of the first and second sea winters in the small spring fish. It will be seen that fish which were of average length at the end of the first sea winter were of average length at the time of capture (end of second sea winter). The average increment in the three groups given in Table 17 is approximately the same.

AGE AND GROWTH OF THE SEA TROUT COMPRISING THE SPRING RUNS IN THIS RIVER.

The material at the authors' disposal was obtained almost exclusively from what might be termed the spring runs of sea trout into the Waterville River and the monthly catches, estimated as a percentage of the catch in the year 1941, have been given in Table 18. In general the average weight (Table 2) decreases as the season progresses. This can be shown to be due to the fact that the proportion of maiden or unspawned fish and, therefore, comparatively light fish, increases with the progress of the season (Table 19). A small proportion (15·7 per cent.) of the total were maiden fish entering the river for the first time, whilst the remainder were previously spawned fish.

Smolt Ages.

Four smolt ages, namely, two, three, four, and five years, were observed, only one five-year smolt being noted. In Table 20 the distribution in the different smolt ages has been given. Two and three-year smolts were almost equal in proportion, and formed together over 90 per cent. of the total.

Age at Maturity and Number of Spawning Marks.

In the previously spawned fish, 28 or 7 per cent. had spawned for the first time in their first post migration winter, i.e. as whitling, 280 or 75 per cent. in the second post migration winter, and 67 or 18 per cent. in the third post migration winter. In general the older smolts tend to return after a short initial feeding in the sea, as will be seen from Table 21. The calculated proportion of the different groups in the maiden fish was as follows. First post migration summer 2 per cent., second post migration summer 45 per cent., third post migration summer 51 per cent. and the fourth post migration summer 2 per cent. There is an apparent discrepancy between the results obtained

from previously spawned and maiden fish. This is due to the fact that the young maiden fish tend to run more abundantly after the close of the netting season, and are, therefore, less liable to capture by the methods employed.

Of the 375 mature fish 119 or 31·7 per cent. had a single spawning mark on their scales, 118 or 31·4 per cent. had two such marks, 97 or 25·9 per cent. three spawning marks, 28 or 7·5 per cent. four spawning marks, and 13 or 3·5 per cent. five spawning marks. Only one of the previously spawned fish had spent more than a year feeding in the sea between what would have been, but for the intervention of man, two successive spawnings.

Condition Co-efficient.

The formula usually adopted by workers using British units of weight and length is that devised by the workers in the Fishery Board for Scotland and used by the late G. H. Nall in his numerous investigations on sea trout (Nall, 1930). This condition co-efficient is calculated according to the formula $K = W/(L^3 \times 0{\cdot}000427)$, where W is the weight in pounds and L is the length in inches. Using this formula Nall found that in good summer condition a mean value of 0·95 was obtained for fish of whitling (first post migration summer) size, and about 1·0 for larger sea trout. In the material at our disposal only a proportion of the fish was weighed owing, as was explained in connection with the salmon of this river, to difficulties experienced in the marketing of the fish. A selection of the fish was weighed, and the condition co-efficients were calculated by means of the above formula. The monthly mean value of the condition co-efficient is given in Table 22.

The mean condition co-efficient ($K = 0{\cdot}9$) is low, but this does not necessarily imply that the sea trout of this river were inferior in edible or sporting characteristics. Just as it has already been shown that there is a considerable variation in the average condition co-efficients of salmon taken in different rivers in Ireland, so with sea trout there is a considerable variation in "condition" from river to river. The spring runs of sea trout into the Waterville River would appear to be slim fish, which may be the result of a very short period feeding in the sea after spawning in the case of over eighty per cent. of the fish.

Whilst dealing with the subject of "condition co-efficient" it is worthy of note that Menzies devised the above formula on the basis of weights and measurements of sea trout from the River Forth in Scotland. Hence the sea trout of the River Forth were used as a standard as were the salmon of the Scottish River Dee. The views expressed by the authors in an earlier section of this paper in connection with the condition co-efficients for salmon may be considered to apply with equal emphasis to the sea trout.

Calculated Lengths.

It is usual in calculating the lengths of sea trout from measurements of the scales, to ignore those fish which have spawned previously. In this case, owing to the very large proportion of mature fish it was decided to measure

all fish, but it might be pointed out that the results obtained from these measurements did not differ materially from those calculated from maiden fish only. There is, therefore, no reason to believe that the results are not fairly reliable.

A. River Life.

The calculated lengths of fish in the different smolt classes are given in Table 23, and a graphical illustration is given in Fig. 3. It is obvious that the fastest growing fish migrated first, resembling in this respect the salmon of most rivers investigated to date. In the spring prior to migration a big proportion of the fish showed rings on the scales corresponding to freshwater

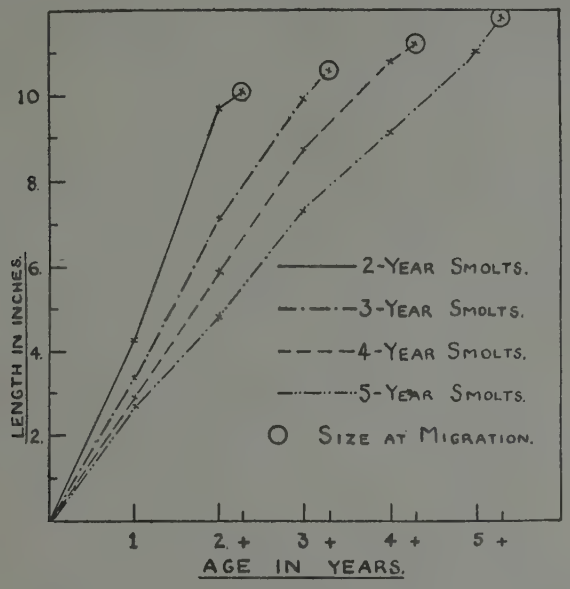

FIG. 3.—Growth curves of the various smolt ages in fresh water for sea trout.

growth. The junction between the portions of the scales representing freshwater and sea growth, respectively, was fairly clearly marked. It was, therefore, possible to calculate the length of the smolt in most cases. In Table 23 the calculated smolt size has been given. Smolts of sea trout (mean length 10·4 inches) of this river are much larger than those of other rivers in Ireland which have been investigated to date. There is a rise in the mean length of the smolt with an increase in smolt age. In Table 24 the calculated lengths in the different groups are given.

B. Sea Life.

Only one fish spawning for the first time in the fourth post migration winter was noted, the other fish all having become mature at least one year earlier (i.e.

by the third post migration winter). In Table 25 the calculated lengths at the end of the first, second, and third post migration winters are given. The average increment made in the first and second years is approximately the same, namely, 3·2 inches and 3·3 inches, respectively.

Average Sizes.

The average sizes are given in Table 26, and in those fish which had spawned for the first time in their second or third post migration winters the average length increased after each return from the sea. Before spawning the average growth in the sea amounted to 3·2 inches per annum, but after spawning the average increase in length between successive spawnings amounted to about half that amount. This is due partly to the fact that the actual period spent feeding in the sea is very much less between spawnings than in the maiden condition.

Oldest and Heaviest Fish.

Two fish (0·5 per cent.) were in their eleventh year since being hatched, 4 (0·9 per cent.) in their tenth year, 31 (7·2 per cent.) in their ninth year and 63 (23·1 per cent.) in their eighth year. The heaviest fish, measuring 30·0 inches in length, weighed 10 lbs. It was, however, somewhat below average condition, even for fish of this river, and would have weighed, in average condition, at least one pound more.

Discussion of Results.

In 1931 the late G. H. Nall published a report on sea trout of the Waterville River, based on 130 sets of scales and data, and his results were very similar to those described herein. The only differences worthy of note were the reduced condition co-efficients for 1941, and reduced percentage of fish in 1941 which had spawned for the first time in their first post migration winter, such differences being well within the range which might be expected from year to year.

The majority of the fish composing the spring runs of sea trout into the Waterville River are mature fish which have spawned in the previous winter and returned to fresh water after a short period feeding in the sea. Considering the very short period which they have spent in the sea in recovering from spawning, new sea growth was quite good, although after the first spawning growth fell off rapidly. Nall (1931) asks the question "why this overwhelming impulse towards a premature return involving a curtailment of growth." As Nall explains, it cannot be because they have a long journey to make, as the Waterville River and its tributaries are short, and the trout have only a short distance to go to the spawning grounds. Competent local observers believe that the sea trout entering the river in the spring are making their way into fresh water, and not merely running up and down with the tides. Indeed at present there is no real tidal portion of the river, as it enters the sea through a storm boulder beach, the pool just inside the mouth being unaffected by the

majority of tides. It is worthy of note that similar spring runs of mature fish are found in the Scottish Ailort which, like the Waterville River, is a short river with the spawning grounds comparatively near to the sea.

The growth rate in the sea, taking all classes of fish together, is higher than that found in most districts on the west coast of Scotland (Nall, 1930), whilst the growth rate in fresh water appears to be higher than in most other rivers investigated. It is possible that the late-running sea trout, which were not sampled, might give somewhat different results from those obtained by consideration of the spring runs. An attempt has been made this year to collect

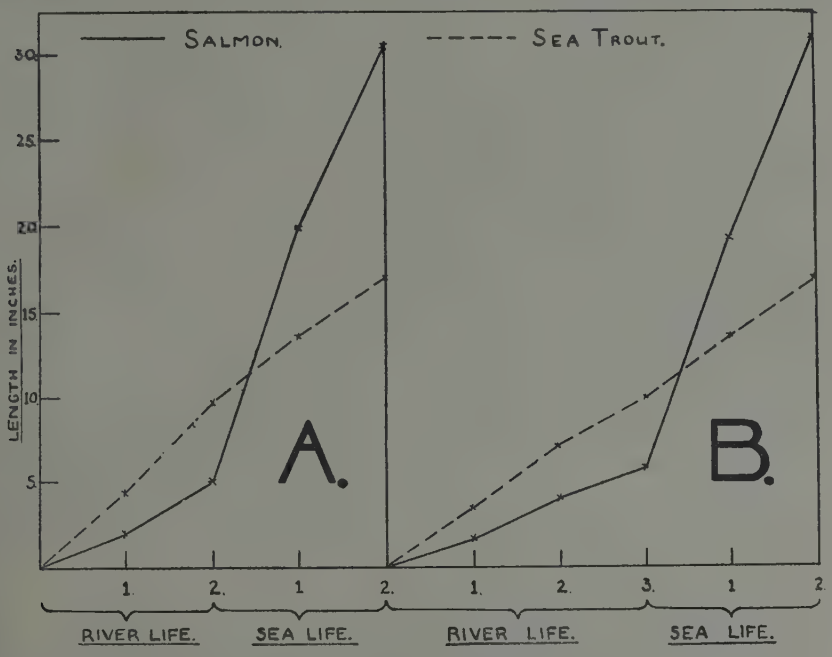

FIG. 4.—Growth curves of salmon and sea trout.
A. Two-year smolt class.
B. Three-year smolt class.

sufficient material to settle the matter, and to obtain a fairly accurate impression of the stocks of sea trout of this river as a whole. It was considered, however, that the results to date were of sufficient interest to include in this report.

A COMPARISON OF THE GROWTH OF SALMON AND SEA TROUT OF THIS RIVER.

The growth rates of salmon and sea trout of this river have been calculated from Tables 14 and 24 and are given in Table 27. A graphical illustration of these growth curves is given in Fig. 4. The salmon grow more slowly in fresh water than the sea trout, and, although the sea trout start their marine life

with a superiority of about four inches over the salmon, at the end of the first sea winter the salmon are approximately six inches longer than the sea trout. In the second sea year the gap between the two species becomes wider, so that the salmon are 13–14 inches longer than the sea trout.

RÉSUMÉ.

(1) A description of the Waterville River and its catchment area is given and it is pointed out that the rocks of the area, belonging to the Old Red Sandstone, contain little or no lime. The waters of this system are either slightly acid or neutral. (Fig. 1 and Table 1.)

(2) Material consisting of sets of scales and data from salmon and sea trout was obtained from the net and weir fisheries on the Waterville River mainly during the year 1941.

(3) *Age and growth of the salmon.*—The bulk (86·0 per cent.) of the fish migrated as two-year smolts (Table 3). Small spring fish (2 winters) and grilse (1 + winters) formed 88·3 per cent. of the total catch (Table 4). The average condition co-efficient was 1·08, spring fish being superior in this respect to summer fish (Table 9). Average sizes in each age group have been given (Table 10). By means of scale measurements the length at end of each year of life in the maiden fish was calculated. In fresh water the fastest growing smolts migrated first (Table 11 and Fig. 2A). Some scales showed a large amount of growth in the spring prior to migration as a smolt. This type of growth was denoted by the term type B smolt growth, whilst, where there was little or no growth in fresh water in the spring prior to migration as a smolt, the growth was termed type A growth. Type A smolts were on the average longer at the end of each year of life in fresh water than type B smolts of the same smolt class (Table 13 and Fig. 2B). An explanation of this phenomenon was given. In the sea the grilse appear to be longer at the end of the first sea winter than any other age group (Table 14). The correlation of the lengths at the end of different years has been given (Tables 15, 16, and 17).

(4) *Age and growth of the sea trout.*—The bulk of the fish migrated as two- and three-year smolts in about equal proportions, a small proportion of four- and five-year smolts being noted (Table 20). Most of the fish were mature fish having entered the river and spawned previously (Table 19). Three-quarters of these fish had spawned for the first time as fish in their second post migration winter, and bore on their scales from one to five spawning marks. In general the fish of this river were of a slim type, the mean condition co-efficient being 0·9 on the Scottish Scale (Table 22). From the length at capture and measurements of the scales, the lengths at the end of the different years of life were calculated (Tables 23 and 24). Smolts of sea trout in this river are much longer than those of any other Irish river investigated. Before spawning the growth rate in the sea amounts to about 3·2 inches per annum, but after maturity this decreases to about half that amount. The average sizes have been given (Table 26). The oldest fish were in their eleventh year, and the heaviest fish measured 30 inches in length and weighed 10 lb.

(5) *Growth of salmon and sea trout compared.*—In fresh water the sea trout had a faster growth rate than the salmon in the two- and three-year smolt classes. Although the sea trout start marine life with an advantage of about four inches over the salmon, at the end of the first sea winter the salmon are approximately six inches longer than the sea trout. The advantage is maintained in the second sea year (Table 27 and Fig. 4).

The collection of the bulk of the material used in this investigation was made under the guidance of one of us (T. S. B.) who also prepared the scales for microscopical examination. The reading of the scales and subsequent working out of the results were carried out in the laboratory of the Fisheries Branch of the Department of Agriculture, Dublin, by the other author.

We wish to take this opportunity to express our thanks to Mr. J. W. Butler, proprietor of the Waterville Fishery, for collecting most of the earlier material and for permission to make the collections during 1941. We are also indebted to Mr. Jeremiah Riordan, of Waterville, who made the necessary collections during 1941.

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APPENDIX (Tables 3 to 17 inclusive refer to salmon, and Tables 18 to 26 inclusive to sea trout).

Table 1. Analyses of waters of Waterville, Cummeragh, Coppal and Finglas Rivers and Lough Currane.

	Waterville River near Salmon Weir	Cummeragh River near Dromod	Coppal River (between Coppal Lough and Lough Currane).	Finglas River	Lough Currane near Church Island
Dissolved Solids					
Total	31.0	33.0	41.0	75.0	37.0
Fixed	2.9	28.0	25.0	61.0	30.0
Volatile	2.0	5.0	16.0	14.0	7.0
Chlorine (Cl)	18.0	10.0	16.0	25.0	19.0
Silica (SiO_2)	1.0	1.0	1.0	8.0	1.0
Iron (Fe_2O_3)	Nil	Nil	Nil	C.1	Nil
Calcium (CaO)	3.0	2.6	2.6	4.4	2.6
Magnesia (MgO)	Trace	Trace	Trace	2.0	Trace
Sodium (Na_2O)	12.2	12.5	12.2	27.5	12.6
Potassium (K_2O)	1.3	1.2	1.3	Trace	1.4
pH	6.3	6.6	6.5	6.3	6.3
Date of Sampling	23/8/1942	21/8/1942	22/8/1942	28/10/1941	23/3/1942

Table 2. Average Weight in lb. of salmon and sea trout taken in the Waterville Fishery in 1941.

Month	Salmon	Sea Trout
1st-15th	11.9 } 11.9	
January 16th-31st	12.0 }	-
February	11.9	-
March	11.8	4.7
April	11.6	3.6
May	10.5	3.4
June	6.9	3.2
July	6.8	3.0
Total	9.9	3.5

Table 3. Percentage of each smolt age in each age group.

Smolt Ages	Age Group (Winters)					Total Maiden Fish
	1+	2	2+	3	With S.M.s	
1	3.2	6.2	5.0	-	13.6	5.1
2	86.6	85.2	95.0	92.0	82.0	86.0
3	10.2	8.6	-	8.0	4.4	8.9
Total	100.0	100.0	100.0	100.0	100.0	100.0

Table 4. Percentage of each age group in the catch of each month.

Month	Age Group (Winters)					Total
	1+	2	2+	3	With S.M.s	
January	-	87	-	4	9	100
February	-	94	-	1	5	100
March	-	95	-	2	3	100
April	-	82	8	4	6	100
May	21	58	13	2	6	100
June	83	8	6	-	3	100
July	83	2	2	-	13	100
Total	32.9	55.4	2.5	2.0	7.2	100

Table 5. Percentages of the total catch of each age group in each month.

Month	Age Group (Winters)					Total
	1+	2	2+	3	With S.M.s	
January	-	28	-	49	24	18.2
February	-	31	-	11	14	18.1
March	-	25	-	22	6	14.8
April	-	7	15	12	4	4.8
May	4	6	29	6	5	5.6
June	42	2	42	-	8	16.9
July	54	1	14	-	39	21.6
Total	100	100	100	100	100	100.0

Table 6. "Absence habit" of previously spawned fish.

Absence Habit	Age at first spawning		Total
	1+ winters	2 winters	
Short (Less than one year)	11	-	11
Long (A full year)	-	32	32
Very long (More than a full year)	1	-	1
Total	12	32	44

Table 7. Showing divided migration and return. The table gives the years in which the fish examined were hatched as percentages of the total for the year.

Returned in 1941 as	Hatched in the year						Total
	1934	1935	1936	1937	1938	1939	
Grilse (1+ winters)	-	-	-	3.1	28.7	1.1	32.9
Small spring fish (2 winters)	-	-	4.7	47.2	3.5	-	55.4
Small summer fish (2+ winters)	-	-	0.2	2.3	-	-	2.5
Large spring fish (3 winters) x	-	0.2	1.8	-	-	-	2.0
Previously spawned fish (With S.M.s)	0.3	4.1	1.0	1.8	-	-	7.2
Total	0.3	4.3	7.7	54.4	32.2	1.1	100.0

x Approximate Values.

Table 8. The estimated size distribution of salmon as percentages of the catch in 1941.

Class Interval in inches xx	Age Group (Winters)					Total
	1+	2	2+	3	With S.M.s	
22	5.46	-	-	-	-	5.46
24	10.91	-	-	-	-	10.91
26	13.20	2.07	0.13	-	0.17	15.57
28	3.12	14.10	0.25	-	0.86	18.33
30	0.21	22.68	0.88	-	1.20	24.97
32	-	13.36	0.62	-	1.20	15.18
34	-	2.21	0.37	0.46	1.71	4.75
36	-	0.98	0.25	0.31	1.20	2.74
38	-	-	-	0.77	0.69	1.46
40	-	-	-	0.31	0.17	0.48
42	-	-	-	0.15	-	0.15
Total	32.9	55.4	2.5	2.0	7.2	100.0

xx Class interval 22 etc. includes all fish between 21.95 and 23.95 inches, etc.

APPENDIX—*continued.*

Table 9. Mean Condition Coefficients of the various age groups.

Age Group	Mean Condition Coefficient	Number Examined
1+ Winters	1.04	38
2 Winters	1.10	240
2+ Winters	1.00	5
3 Winters	1.13	8
With S.M.s	1.13	20
Total	1.08	311
All spring fish	1.10	267
All summer fish	1.05	44

Table 10. Mean lengths and estimated mean weights in the various age groups.

Age Group	Mean length in inches.	Estimated mean weight in lb.
1+ Winters	25.4	5.9
2 Winters	30.9	11.7
2+ Winters	31.8	11.6
3 Winters	38.1	22.7
With S.M.s	34.5	16.7

Table 11. Calculated lengths in inches in the various smolt classes in fresh water.

Smolt Age	Number Examined	Mean length at end of			Mean Smolt Length
		1st year	2nd year	3rd year	
1	33	3.2	-	-	5.2
2	553	2.0	5.0	-	5.7
3	56	1.7	4.0	5.7	6.0

Mean length of all smolts = 5.7 inches.

Table 12. Distribution of the two smolt types in the various smolt classes as percentages of the total catch of maiden fish.

Smolt Age	Type A smolts	Type B smolts	Total
1	-	5.1	5.1
2	13.4	72.6	86.0
3	5.0	3.9	8.9
Total	18.4	81.6	100.0

Table 13. Calculated mean lengths in inches in Type A and Type B smolts of the various smolt classes.

Smolt Age	Type A smolts					Type B smolts				
	Number Examined	Length at end of			Mean Smolt Length	Number Examined	Length at end of			Mean Smolt Length
		1st winter	2nd winter	3rd winter			1st winter	2nd winter	3rd winter	
1	-	-	-	-	-	33	3.2	-	-	5.2
2	70	2.3	6.2	-	6.2	483	1.9	4.8	-	5.6
3	32	1.7	4.1	5.8	5.8	24	1.6	3.8	5.5	6.3

Table 14. Calculated mean lengths in inches at end of each winter of River and Sea Life.

Age Group	Number Examined	River Life				Sea Life			
		Mean length at end of			Mean Smolt Length	Mean length at end of			Mean Length at Capture.
		1st winter	2nd winter	3rd winter		1st winter	2nd winter	3rd winter	
1.1+	5	3.3	-	-	5.3	20.5	-	-	26.8
2.1+	135	2.1	5.0	-	5.6	20.2	-	-	25.2
3.1+	16	1.7	4.0	5.8	6.2	20.1	-	-	25.1
1.2	27	3.2	-	-	4.9	19.0	30.9	-	30.9
2.2	389	2.0	5.0	-	5.5	19.4	30.7	-	30.7
3.2	39	1.7	4.0	5.7	5.9	19.3	31.0	-	31.0
1.2+	1	3.3	-	-	5.8	19.5	31.7	-	33.0
2.2+	18	1.8	4.8	-	5.7	18.5	29.7	-	31.8
2.3	11	2.0	5.2	-	5.8	19.7	31.2	38.2	38.2
3.3	1	1.0	3.0	4.4	5.2	19.5	31.4	38.0	38.0

Table 15. Correlation between lengths at end of first and second winter in the two-year smolt class.

Length at end of 1st winter	Number examined	Mean length at end of 2nd winter	Mean Increment made in 2nd year
0.95-1.45 ins.	74	3.9 inches	2.7 inches
1.45-1.95 ins.	188	4.5 inches	2.8 inches
1.95-2.45 ins.	182	5.3 inches	3.1 inches
2.45-2.95 ins.	85	5.8 inches	3.1 inches
2.95-3.45 ins.	21	6.6 inches	3.4 inches
3.45-3.95 ins.	2	7.7 inches	4.0 inches

Table 16. Correlation between smolt length and length at end of first sea winter in the grilse and small spring fish.

Smolt length	Grilse		Small spring fish	
	Number examined	Mean length at end of first sea winter	Number examined	Mean length at end of first sea winter
Less than 4.95 ins.	27	18.9 ins.	87	18.2 ins.
4.95-5.95 ins.	65	20.0 ins.	191	19.2 ins.
More than 5.95 ins.	64	21.3 ins.	171	20.8 ins.

Table 17. Correlation between lengths at end of first and second sea winters in the small spring fish (2 winters age group).

Length at end of first sea winter	No. examined	Mean length at end of second sea winter	Mean increment in second sea winter
Less than 18.95 ins.	177	29.0 ins.	11.1 ins.
18.95 - 19.95	131	30.9 ins.	11.4 ins.
Above 19.95 ins.	146	32.4 ins.	11.2 ins.

Table 18. Monthly catches, as percentages of the total catch for the year 1941.

Month	Percentage Monthly Catch
(February	
(March	7.2%
April	27.6%
May	31.6%
June	30.5%
July	3.1%
Total	100.0%

Table 19. Monthly proportion of maiden and previously spawned fish.

Month	Maiden Fish	Previously spawned fish
(February		
(March	10%	90%
April	7%	93%
May	17%	83%
June	19%	81%
July	62%	38%
Total	15.7%	84.3%

APPENDIX—*continued.*

Table 20. Proportion of the different smolt ages.

	Smolt age				Total
	Two-year	Three-year	Four-year	Five-year	
Number of Individuals	201	198	33	1	433
Percentage of Total	46.5%	45.7%	7.6%	0.2%	100.0%

Table 21. Age at first spawning in the different smolt classes.

Age at first spawning	Smolt Age								Total	
	2		3		4		5			
	No.	%	No.	%	No.	%	No.	%	No.	%
First Post-Migration year	6	4	13	7	8	29	1	100	28	7
Second Post-Migration year	134	77	129	74	18	64	-	-	281	75
Third Post-Migration year	32	19	32	19	2	7	-	-	66	18
Total	172	100	174	100	28	100	1	100	375	100

Table 22. Mean monthly condition coefficients.

Month	Number examined	Mean Condition Coefficient
February March	31	0.89
April	115	0.92
May	60	0.85
June	32	0.92
July	4	0.88
Total	242	0.90

Table 23. Calculated mean lengths in inches in fresh water.

Smolt Age	Number	Mean length at end of					Mean Smolt length
		1st Winter	2nd Winter	3rd Winter	4th Winter	5th Winter	
2	199	4.3	9.7	-	-	-	10.1
3	189	3.4	7.1	9.9	-	-	10.6
4	32	2.9	5.9	8.7	10.8	-	11.2
5	1	2.7	4.8	7.3	9.1	11.0	11.8

Table 24. Calculated mean lengths in inches at the end of each year of life in the river and sea.

Group	Number Examined	River Life						Sea Life		
		Mean length at end of					Mean Smolt Size	Mean length at end of		
		1st winter	2nd winter	3rd winter	4th winter	5th winter		1st winter	2nd winter	3rd winter
2+	7	4.5	9.3	-	-	-	9.9	-	-	-
3+	11	3.7	8.1	10.9	-	-	11.4	-	-	-
4+	7	3.1	6.2	9.1	11.4	-	11.9	-	-	-
5+	1	2.7	4.8	7.3	9.1	11.0	11.8	-	-	-
2.1+	145	4.3	9.8	-	-	-	10.1	13.7	-	-
3.1+	134	3.4	7.2	9.8	-	-	10.6	13.4	-	-
4.1+	20	2.9	5.8	8.7	10.8	-	11.0	14.3	-	-
2.2+	46	4.3	9.3	-	-	-	10.2	13.3	16.9	-
3.2+	44	3.2	6.6	9.7	-	-	10.4	13.7	16.9	-
4.2+	5	2.7	5.9	8.4	10.4	-	11.0	14.7	17.5	-
2.3+	1	3.1	9.2	-	-	-	10.5	13.0	15.2	18.1

Table 26. Average sizes in the different age groups. Mean weight in last column being calculated on basis of K = 0.9.

Age Group	Number examined	Length in inches			Mean Weight
		Minimum	Maximum	Mean	
.+	1	-	-	12.0	0.7
.+S.M.+	3	-	-	18.7	2.5
.+2S.M+	8	20.0	23.5	21.7	3.8
.+3S.M.+	8	20.0	25.0	21.7	3.8
.+4S.M.+	5	20.9	24.5	22.3	4.3
.+5S.M.+	3	20.9	23.2	22.1	4.1
.1+	27	13.5	20.0	17.1	1.9
.1+S.M.+	76	14.5	24.5	19.1	2.7
.1+2S.M+	97	18.5	25.0	21.5	3.8
.1+3S.M+	77	18.2	25.5	22.7	4.5
.1+4S.M+	23	20.9	25.0	23.4	4.9
.1+5S.M+	6	23.1	26.0	24.6	5.7
.1+S.M.I+	1	-	-	24.0	5.3
.2+	30	17.5	24.0	20.6	3.4
.2+S.M.+	38	19.0	25.0	22.2	4.3
.2+2S.M+	13	21.0	25.5	22.9	4.6
.2+3S.M+	12	22.0	30.0	24.4	5.6
.2+5S.M+	4	24.0	27.0	25.3	6.2
.3+	1	-	-	19.5	2.8
Total	433	12.0	30.0	-	-

Table 25. Calculated mean lengths in inches in the sea.

Age Group	Number examined	Mean length at end of		
		1st winter	2nd winter	3rd winter
2nd Post Migration Summer	299	13.6	-	-
3rd Post Migration Summer	95	13.6	16.9	-
4th Post Migration Summer	1	13.0	15.2	18.1
Total	395	13.6	16.9	18.1

Table 27. Growth rates of salmon and sea trout in the two-and three-year smolts.

Type of Fish	Calculated mean lengths in inches				
	River			Sea	
	1st year	2nd year	3rd year	1st year	2nd year
Two-year Smolt Class					
Salmon	2.0	5.0	-	13.3	30.5
Sea Trout	4.3	9.7	-	13.6	16.9
Three-year Smolt Class					
Salmon	1.7	4.0	5.7	13.5	31.0
Sea Trout	3.4	7.1	9.8	13.5	16.9

No. 10.

ASCORBIC ACID.

Part I: Detection and Estimation.

By W. R. FEARON and EINHART KAWERAU.

[Read June 23, 1942. Published separately April 16, 1943.]

The present inadequate supply of imported citrus fruits and the scarcity of many home-grown fruits have led to the belief that the Irish national dietary may be exposed to the dangers of a deficiency in vitamin C. For this reason a survey has been undertaken, with the assistance of a grant from the Medical Research Council of Eire, the object being to explore and assess available sources of supply. In addition, a study has been made of the stability of the vitamin, especially with regard to the effects of storage, cooking, and industrial treatment. The first part of the work now submitted deals with the methods available for the detection and estimation of vitamin C, and describes new reactions for the detection of both the reduced and the oxidised form of the vitamin.

Vitamin C, as is generally known, can occur in three forms: (1) free ascorbic acid, (2) oxidised or dehydro-ascorbic acid, and (3) "bound" ascorbic acid, the nature of which is somewhat obscure. All three forms are biologically equivalent in that they are interchangeable in human nutrition, but the fact that they differ in their chemical properties introduces complications into the procedure for estimating the vitamin content of natural food materials. Thus, when dealing with plant or animal tissue, it is customary to extract with an acid solvent in order to liberate any "bound" ascorbic acid that may be present. The extract is then treated with hydrogen sulphide to reduce any dehydro-ascorbic acid present, and the resulting solution of ascorbic acid is then titrated by means of an appropriate indicator. Many errors may arise in this procedure.

The outstanding characteristic of ascorbic acid is its ability to act as a powerful reducing agent, or hydrogen-donor, even in strongly acid solutions. This property is revealed by the rapid reduction of various reagents used from time to time by various workers for the detection of the vitamin. These reagents are represented by: (1) Ag^{+} in acid solution (Szent-Györgyi, 1928), (2) Cu^{++} (Szent-Györgyi, 1928), (3) Au^{+++} (Emmerie, 1935), (4) Fe^{+++} (Tauber and Kleiner, 1935; McFarlane, 1936), (5) Hg^{++} (Pittarelli, 1936), (6) V_2O_5 (Freses, 1938), (7) H_2SeO_3 (Emmerie, 1935), (8) Ceric acid (Süllman, 1938), (9) Tungstic acid (Medes, 1935; Fujita *et al.*, 1935, 1938; Shinohara

and Padis, 1936), (10) Molybdic acid (v. Euler and Burström, 1936), (11) Iodine (Szent-Györgyi, 1927, 1928, (12) Dibromophenol-indophenol (Tillmans *et al.*, 1932), (13) Dichlorophenol-indophenol (Tillmans *et al.*, 1932), (14) Methylene blue in presence of light (Martini and Bonsignore, 1934; Lund and Lieck, 1936; Gál, 1936), (15) Cacothelin (Rosenthaler, 1938), (16) Dichlorobenzene-indophenol (Morell, 1941). To this list we now add (17) *o*-dinitrobenzene, which we find is rapidly reduced in alkaline solution by the vitamin, yielding a violet pigment.

The *o*-Dinitrobenzene Test for Ascorbic Acid.

To 5 ml. of the vitamin solution add 2–4 drops of a saturated aqueous solution of *o*-dinitrobenzene, followed by 20 drops of 20 per cent. sodium hydroxide. If more than 1·5 mg. of ascorbic acid be present, a violet colour develops in a few seconds, and reaches maximum intensity in about 10 minutes at ordinary temperature. In weaker solutions colour development is proportionately slower, the rate depending on the temperature and degree of alkalinity. Under the above conditions, the reaction is not given by dehydro-ascorbic acid, glutathione, cysteine, creatinine, or uric acid. The reducing sugars eventually give the reaction, but only when in much higher concentrations and after remaining for at least half-an-hour in contact with the reagent. If the mixture is heated it loses its selectivity, and many reducing agents give the reaction. The violet pigment has been isolated and identified as the salt of the nitroxylic acid formed by simple reduction of the dinitrobenzene (Fearon and Kawerau, 1943).

Dehydro-ascorbic Acid.

Dehydro-ascorbic acid, the oxidised form of vitamin C, is usually detected indirectly by conversion to ascorbic acid. Szent-Györgyi (1928) was the first to show that this could be quantitatively effected by hydrogen sulphide, and by this means dehydro-ascorbic acid has been shown to occur in many plant and animal tissues (Bessey and King, 1933; Van Eeklen *et al.*, 1934; Bacharach *et al.*, 1934; Gabbe, 1934). Other methods of reduction have been advocated, such as stannous chloride (Roe, 1936). Our own observations indicate that neither magnesium amalgam nor aluminium amalgam is effective, and Barron *et al.* report similar negative results from the use of nascent hydrogen in presence of palladium (1935). A serious source of error following the use of hydrogen sulphide has been observed by King (1941), who has shown that many naturally-occurring aldehydes, ketones, and quinones are converted by hydrogen sulphide into compounds that reduce the titration indicators in a manner similar to ascorbic acid.

The actual amount of dehydro-ascorbic acid originally present in a tissue is also somewhat uncertain, since catalytic oxidation of the vitamin is liable to occur at some stage in the analytical treatment. This is usually due to

copper either in ionic form or associated with a protein, such as the so-called "ascorbic oxidase" enzyme. Barron *et al.* (1935) have shown that 46 micrograms of copper per litre are capable of effecting a 50 per cent. oxidation of the ascorbic acid present, in six hours at pH 1·07.

Copper may be introduced into the system in three ways: (1) by the tissue itself; (2) by the distilled water used in making the extracts and preparing the reagents; and (3) by the actual reagents. By the use of glass-distilled water and specially purified reagents, copper-contamination can be lessened, but the influence of copper derived from the tissue can only be overcome by the use of a reactant that will depress the ionisation of the metal. Metaphosphoric acid has been found to be effective for this purpose (Fujita and Iwatake, 1935), and is widely employed. Copper immobilisation also inhibits the oxidation of ascorbic acid by enzymes, since it forms part of the prosthetic group of the oxidase system (Kubowitz, 1937, 1938; Lovett-Janison and Nelson, 1940; McCarthy *et al.*, 1939; Meiklejohn and Stewart, 1941).

Only one direct test for dehydro-ascorbic acid appears to have attracted attention up to the present. On treatment with 2:4-dinitro-phenylhydrazine an osazone is formed, which can be separated and estimated gravimetrically or by titration with titanium chloride, or colorimetrically by the red colour it yields when dissolved in concentrated sulphuric acid (Roe and Kuether, 1942).

The Acid Test for Dehydro-ascorbic Acid.

When a solution of dehydro-ascorbic acid is buffered to the region of pH 4 and gently boiled, we have observed that it develops a stable grass-green colour. The test is not given by ascorbic acid, or by any of the familiar biological acids, sugars, proteins, and related compounds. The test may be demonstrated by carefully oxidising 5 ml. of dilute (0·01–0·001 per cent.) ascorbic acid by addition of one per cent. iodine solution. Any excess of the halogen must be removed by addition of more ascorbic acid or a particle of thiourea. The mixture, before or after oxidation, is set to pH 3–4 by addition of about 5 g. of sodium acetate and 10 drops of glacial acetic acid. On boiling for a couple of minutes the characteristic green colour appears, and may be diluted with water for the purpose of colorimetric comparison. The possible mechanism of the test is discussed elsewhere (Fearon and Kawerau, 1943).

"Bound" Ascorbic Acid.

Fujita and Ebihara (1939) conclude that some at least of the ascorbic acid present in tissues occurs as a "bound" form, that can be released by metaphosphoric acid extraction or by boiling, provided that the destructive effects of copper and oxidising enzymes are properly controlled. This conclusion appears to have been misapprehended by Harris and Olliver (1942) in their recent review. The increased yield of indophenol-reducing material obtained by

boiling has also been noted by Mack and Tressler (1937), and by Borsook *et al.* (1937), and has been attributed to formation of diketo-gulonic acid, a degradation product of ascorbic acid that is more strongly reducing than the parent compound. Harris and Olliver report their complete failure to find evidence for the existence of "bound" ascorbic acid in vegetable material.

"Bound" ascorbic acid has also been reported to occur in urine (Scarborough and Stewart, 1937; Guha and Sen-Gupta, 1938, 1940). Holts and Walter (1940) postulate a protein-bound ascorbate, which can be released by acid or enzyme (pepsin or papain) hydrolysis. We have some indications that, in the potato, dehydro-ascorbic acid can exist in a complex form less susceptible to oxidative destruction than either of the free forms of the vitamin.

Estimation of Ascorbic Acid.

Application of the new tests to biological material leads us to agree with the conclusions of Harris and Olliver (1942), Stone (1937), and Bessey (1938), all of whom maintain that most of the vitamin C of the dietary, in as far as it is derived from fresh foodstuffs, consists of free ascorbic acid in the reduced state. The tests that depend on the reducing potency of the tissue extracts and products are hence of primary importance, and we have examined their quantitative application to the assay of the vitamin.

1. *Silver*: Although the metallic ion is very readily reduced by traces of ascorbic acid, it is not possible to titrate either reactant by means of the other, since there is no sharp end-point obtainable. The reduced silver appears in a colloidal form, and obscures the titration when carried out in presence of adsorption indicators, such as tartrazol, even when back-titrated with standard chloride solutions at varying degrees of acidity.

2. *Copper*: Ascorbic acid at room temperature rapidly reduces the alkaline sugar reagents, such as those of Fehling or Benedict. But, as in the silver solutions, the end-point is not sharp, and there is a considerable lag in the final stage of the reaction.

3. *Tungstic Acid and Molybdic Acid*: In a series of papers, Fujita and his colleagues (1935, 1937, 1938) discuss the reduction of phospho-tungstic and phosphomolybdic acids by ascorbic acid, and conclude that the reagents employed by earlier workers (Bezssonoff, 1935; Euler and Burström, 1936) are not selective enough to be of value in the estimation of small quantities of the vitamin. They also found that Folin's molybdic reagent, at first thought to be suitable, has the defect that the blue pigment it yields with the vitamin fades, even at freezing-point, and thus makes colorimetric work untrustworthy. Since alkali is used in the development of these pigments the method has the further disadvantage of being inapplicable to vegetable extracts rich in flavones, which give intense yellow colours on addition of alkali.

Fujita and Ebihara (1938) overcome this difficulty by using the Folin reagent at pH 3, and destroying non-ascorbic reducing substances by means of iodoacetic acid. The method, however, requires the use of the photo-metric colorimeter, and specially prepared filters.

4. *Methylene Blue*: This dye has been advocated as more selective for ascorbic titration than the indophenols (Martini and Bonsignore, 1934; Wachholder and Podesta, 1936), since it has a lower redox potential, and therefore is less affected by non-ascorbic reducing substances, especially those of the thiol class. Gál (1936) and Lund and Trier (1939) have devised apparently satisfactory methods based on the bleaching of methylene blue, but Armentanó (1940), after a critical survey of the subject, concludes that the titration is so sensitive to copper and iron ions that only a rigid control of the pH and other conditions can exclude errors due to re-oxidation of the leuco-form of dye by atmospheric oxygen.

The methods finally adopted in the present work were: (1) ferric titration for ascorbic acid in phosphate-free solutions, (2) iodine titration for ascorbic acid in biological concentration of more than 10 mg. per 100 ml., and (3) dichlorophenol-indophenol titration for ascorbic acid in concentrations less than 10 mg. per 100 ml.

Estimation of Ascorbic Acid by Ferric Titration.

This method is based on the quantitative reduction of Fe^{+++} to Fe^{++} by ascorbic acid in acid solution, thiocyanate being used as an internal indicator. Its application to biological material is limited by the fact that phosphates interfere by precipitating Fe^{+++}; apart from this, the method is suitable for studying the course and rate of ascorbic oxidation in presence of various catalysts.

Reagents: 1. Standard Iron solution containing 0·1 mg. Fe^{+++} per litre, prepared by dissolving 0·702 g. of analytically pure ferrous ammonium sulphate in 100 ml. of distilled water. Five ml. of sulphuric acid diluted 1 in 5 are added. The iron is then oxidised by exact addition of 0·2 per cent. potassium permanganate until a faint pink colour persists. The solution is then diluted to 1 litre and well mixed. 2. Indicator, 1 per cent. potassium thiocyanate.

Method: An appropriate quantity of solution for analysis, 1 to 10 ml., is acidified with a few drops of glacial acetic acid. One ml. of indicator is added, and the mixture titrated with the standard iron solution from a micro-burette until a definite pink shade persists. At 20° C. the speed of titration is rapid almost up to the end-point. On the assumption that 2 atoms of iron are reduced by one molecule of ascorbic acid, 1 ml. of standard iron solution (= 0·1 mg. Fe^{+++}) is equivalent to 0·157 mg. of ascorbic acid. This value is five times less than the corresponding ascorbic equivalent of the N/100 iodine used in the alternative method, and thus the iron titration is better suited for estimation of small amounts of vitamin C in absence of phosphate interference.

Estimation of Ascorbic Acid by Iodine Titration.

Working with suitable material, iodine has been shown to yield good results in ascorbic acid estimations (Szent-Györgyi, 1928; Ballentine, 1941). The reagent (N/100 Iodine) is easily standardised against thiosulphate, and is

much superior in keeping qualities to dichlorophenol-indophenol. Its chief disadvantage is due to its reduction by thiol compounds, notably glutathione, for the estimation of which it has frequently been used, and these compounds should be absent from the extracts being assayed for vitamin C. The -SH group in biological thiols can be rendered inactive by addition of heavy metals, such as Hg^{++}, but these ions are liable to catalyse the oxidation of ascorbic acid, or combine with it to form salts. Iodine also tends to be adsorbed by proteins or polysaccharides present in the extracts, and thus give unduly high titration values. Using a 1 per cent. solution of soluble starch as indicator, we have observed that 2 drops added to 1 ml. of titration substrate are capable of adsorbing iodine to the equivalent of 0·02 mg. of ascorbic acid. This effect makes it difficult to titrate a colloidal solution such as milk, and the method is useful chiefly for the estimation of ascorbic acid in simple solutions and colourless fruit juices. Where the extracts are pigmented, the bleaching of the iodine may not be observable, and the starch indicator is obscured. To overcome these limitations, we have used xylene or benzene as a partition solvent, whereby a trace of free iodine in the completed titration can be detected. In practice, about 0·5 ml. of xylene is added to the solution being titrated. After each addition of iodine, the tube is shaken gently, and the xylene allowed to separate out. The end-point is shown by the appearance of a pink colour in the xylene layer. The method is sensitive, and will reveal one drop of N/1000 iodine added to 10 ml. of water. Natural fruit pigments and the dyestuffs commonly employed to colour preserves are not extracted by xylene, so the method is widely applicable to analyses of commercial products. The utility range of the method extends down to ascorbic values of about 10 mg. per 100 ml.

Estimation of Ascorbic Acid by Dichlorophenol-Indophenol.

Dichlorophenol-indophenol is a highly sensitive titration reagent for ascorbic acid. The optimum conditions have been exhaustively worked out (Tillmans *et al.*, 1932; Birch *et al.*, 1933; Harris and Olliver, 1942), and we have nothing new to add. It is the most suitable method when dealing with ascorbic concentrations below 10 mg. per 100 ml., or whenever the iron or iodine methods are inapplicable.

Although the dye is bleached by glutathione and by cysteine, even in solutions as acid as pH 3, the rate of reduction by these compounds is much slower than reduction by ascorbic acid, and, by rapid titration, consistent values for the vitamin are obtainable with practice.

Unfortunately, the dye is very unstable in aqueous solution, and must be standardised every time it is used. This may be done by means of pure crystalline ascorbic acid, a reagent not readily obtainable, or by standardisation in bulk, using other reducing agents (Menaker and Guerrant, 1938). Ramsey and Colichman (1942) avoid the need for an accurately standardised dye by a double titration method, involving the use of iodine. We have endeavoured to

develop this method, and the results will be submitted in a later communication.

For the purpose of checking the strength of the solutions prepared from the tablets of dyestuff now on the market, we have made use of our observation that the colour of dichlorophenol-indophenol in acetic acid solution can be very closely matched by thymol blue in a glycerol solution at pH 2·0.

Where the solution being titrated is already pigmented, chloroform, as suggested by McHenry and Graham (1935), can be used to extract the dye, and thus reveal the colour change at the end-point. Chloroform, however, is a solvent for some natural pigments, and, when these are present, other more selective solvents, such as a mixture of xylene and amyl alcohol, may be used (Lanke, 1939).

ESTIMATION OF DEHYDRO-ASCORBIC ACID.

Preliminary work shows that it is possible to estimate dehydro-ascorbic acid in unpigmented extracts by means of the colour reaction obtained in acid solutions. The green product can be accurately matched in a colorimeter by a standard solution of uranium acetate or nitrate, and obeys the Beer-Lambert law over a sufficiently wide range of concentration to cover the requirements of ordinary biological analyses.

SUMMARY.

1. The available methods for the detection and estimation of ascorbic acid, dehydro-ascorbic acid, and "bound" ascorbic acid are classified and discussed.

2. A new colour test for ascorbic acid is described, depending on the reduction of *o*-dinitrobenzene in alkaline solution at room-temperature.

3. A new colour test for dehydro-ascorbic acid is described, depending on the formation of a green pigment when the vitamin is boiled in solution buffered to pH 3–5.

4. A method for the estimation of ascorbic acid by ferric titration, using thiocyanate as indicator, is described.

5. A method for the estimation of ascorbic acid by iodine titration, using xylene as a partition indicator, is described.

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No. 11.

ON AN EPIDEMIC OF *GIBBERELLA SAUBINETII* (Mont.) Sacc. ON WHEAT IN EIRE IN 1942.

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PLATES 4 AND 5.

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INTRODUCTION.

IN Great Britain and Ireland the disease known as "Ear Blight" of cereals is one in which the spikes or panicles are attacked by a fungus that forms more or less a weft of mycelium on affected spikelets and interferes with the normal development of the grain. The term "Ear Blight," however, refers to the effects of a number of closely similar species of *Fusarium*. Most of these cannot be recognized specifically from a mere superficial examination of diseased heads or grain, and very often the actual species involved can only be identified after considerable cultural work in the laboratory. A search of the records kept during the past twenty years in this country shows that, in the case of "Ear Blight" of wheat, while *Fusarium* spp. (representing unidentified species) have occasionally been recorded, the species usually associated with this disease have been *F. avenaceum*, *F. herbarum*, and *F. culmorum*. To these three, a fourth, namely, *Fusarium graminearum* Schwabe (*Gibberella Saubinetii* (Mont.) Sacc.[1]), must now be added. As Lafferty found this organism on wheat grain in 1918 in Ireland, it probably figured amongst the unidentified species during the past twenty years, but in the absence of cultural work it escaped recognition.

In attacks due to the four organisms, *Fusarium avenaceum*, *F. herbarum*, *F. culmorum* and *F. graminearum* Schwabe, the symptoms of disease on wheat ears are all alike. Each of these organisms gives rise to a mycelium which varies from dull or faint pink to carmine in colour. Affected grains are often similarly coloured, either partially or wholly, the embryo end of the grain generally showing the greatest development of red pigment. Though *F. graminearum* (*G. Saubinetii*) is stated to be the most common cause of reddish discoloured wheat grains, according to Atanasoff (2) other fungi may

[1] According to Petch the correct name of the fungus causing "Scab" on cereals is *Gibberella Zeae* (Schwein.) Petch. For full discussion on this question of nomenclature see Petch, T.—Ann. Mycol., Berl., 34, pp. 256–260, 1936. In the absence of general acceptance of Petch's claim the present writer prefers to adhere to *Gibberella Saubinetii* (Mont.) Sacc., the name by which the fungus is most widely known.

occasionally be responsible for it, and reddish discoloured grains are not, therefore, indicative of any single organism. In *Fusarium avenaceum*, *F. herbarum*, and *F. culmorum* asexual fructifications alone occur, being produced both on diseased grain and in pure cultures, but *Fusarium graminearum* Schwabe is recognized as being simply the conidial stage of the fungus *Gibberella Saubinetii* (Mont.) Sacc. Hence, the production of typical perithecia containing the characteristic asci and ascospores is the diagnostic feature in this case.

While attacks of an orange-coloured fungus on wheat ears were first described in England in 1884, being reported as a new disease under the name *Fusisporium culmorum* W. Sm. (30), and other species of *Fusisporium* (*Fusarium*) were even then known to occur on various cereals, it was not until 1930 that the fungus *Gibberella Saubinetii* (Mont.) Sacc. was definitely recorded on British cereals. Bennett (4) isolated this fungus from discoloured wheat grains in 1928, and on incubation of some of the naturally infected grain early in the following year he obtained perithecia, a number of which eventually developed asci and ascospores. Bennett also obtained perithecial development on artificially inoculated wheat grains brought to maturity on sterile soil. The same worker afterwards isolated *Gibberella Saubinetii* from bases of barley, oats, wheat, rye, and *Triticum monococcum* plants, and showed that, though the fungus was widespread throughout England, the climatic conditions there were not favourable for perithecial formation (5). Finally, however, Bennett (6) reported the fortuitous occurrence of these fructifications on the ears of Little Joss wheat in 1932, this being the first record (and apparently up to the present time the only one) of perithecial development in England under natural conditions.

In addition to Bennett's papers (4), (5), (6) on *G. Saubinetii*, the only other reference to the occurrence of this fungus on cereals in Great Britain is that by Dennis and Foister (9), where it is listed as occurring on wheat in Scotland.

Bennett in one of his papers (5) refers to an unpublished record by Lafferty of *G. Saubinetii* on wheat in Co. Galway, Ireland, in 1918. From information supplied to the writer verbally by Dr. Lafferty, it now transpires that during a study of *Fusarium* species at that time, he isolated *F. graminearum* from discoloured wheat grains, and later obtained perithecia in pure cultures of the organism, thus settling the question of identification. This isolation of the fungus by Dr. Lafferty is the only previous authentic evidence of the presence of *Gibberella Saubinetii* in this country, but it has doubtless been endemic in many districts for a long time.

Though the number of publications in Great Britain and Ireland on *Gibberella Saubinetii* on cereals is small, the fungus has been intensively studied in other countries, particularly in America. The importance of this organism lies not only in the actual loss sustained by the grower through "Ear Blight," and to a less extent through "Seedling Blight," but also to the well-known toxic effect both of diseased grain and of the manufactured products.

The present paper is an account of an epidemic of *Gibberella Saubinetii* (mainly on wheat) in Eire in 1942, together with some details of actual losses incurred. The latter feature of the disease is one on which practically no information has hitherto been available in these islands.

The Occurrence of *Gibberella Saubinetii* on Wheat in Eire in 1942.

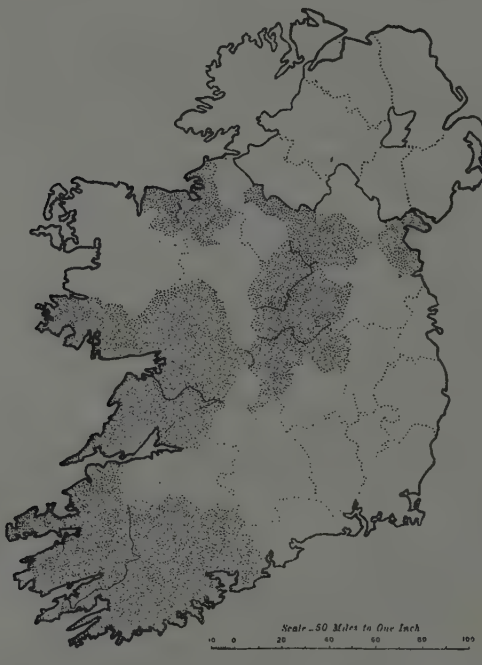
Fungus attacks on wheat ears, due to a species of *Fusarium*, were observed in several localities in Eire in 1942, the disease being present from the flowering time of the host onwards. As the season advanced, complaints from growers became numerous when it was seen that wheat ears were not filling out properly, and by harvest time it was obvious that serious losses had occurred in certain districts. A couple of examples from different counties will suffice to show what was taking place.

On 28th August, diseased wheat specimens, accompanied by a report, were received from Co. Kerry, the correspondent stating that "the crop from which the sample was taken was not worth cutting and that many crops of wheat in his district were similarly affected." The fungus attack on the wheat heads received was severe, and while certainty about the correct identification of the *Fusarium* species present was not then possible, later, on receiving further specimens from the same crop, the fungus was identified as *Fusarium graminearum* Schwabe, the conidial stage of *Gibberella Saubinetii*. This was afterwards confirmed when many heads of wheat with mature perithecia of the fungus were received from the correspondent. The second example was from the midlands. On the 23rd September, Mr. P. Conroy, Agricultural Instructor, Co. Westmeath, forwarded a few wheat ears for the identification of a fungus which was forming black incrustations on the spikelets. The black crusts on the wheat ears in this case proved to be composed largely of mature perithecia of *G. Saubinetii*. In answer to an enquiry regarding the prevalence of the disease on the crop, with a request for further specimens and information concerning the outbreak, Mr. Conroy kindly sent some sixty wheat heads with perithecia on them, and also supplied the following observations:—"The pink *Fusarium* stage of the fungus was prevalent throughout the crop, and black spotted heads with perithecia occurred to the extent of one ear to every two square yards over the whole area, which was slightly above two statute acres. The wheat ears did not turn down in the characteristic manner of a full crop. The workmen commented on the peculiar taste of the grain, the lightness of the sheaves, and the absence of grain-feel in the ears."

As other specimens with perithecia of *G. Saubinetii* and several samples of pink discoloured grain came to hand, the co-operation of the Department of Agriculture was sought in order to ascertain to what extent the disease was distributed throughout the country. Unfortunately, the Department was not approached until the season was well advanced, many of the wheat crops were

already harvested, and a considerable number had been threshed. The Department, however, immediately sent out a memorandum to all the Agricultural Instructors in the various counties of Eire, asking that any wheat (either in grain or in ear) bearing evidence of this disease might be forwarded for examination. In the case of grain already threshed, a sample of the chaff was requested, if available, and attention was drawn to the blackening of the glumes with an appearance similar to smut. Information was also requested regarding (*a*) variety of wheat affected, (*b*) seed dressing, if any, before sowing, and (*c*) particulars of previous cropping.

As a result of this memorandum, many samples of diseased wheat were received, some showing only the *Fusarium* stage, but others displaying the perfect fructifications. Specimens of wheat with mature perithecia of the fungus *Gibberella Saubinetii* have now been received from the following



Map of Eire, shaded areas are counties from which perithecia of *Gibberella Saubinetii* have been recorded in 1942.

counties:—Louth, Cavan, Sligo, Galway, Clare, Kerry, Cork, Westmeath, Longford, and Offaly. Therefore, the disease is widespread throughout Eire, as can be seen from a glance at the accompanying map.

As the criterion for the identification of the fungus was the presence of typical perithecia containing asci and ascospores, and the investigation was

started too late in the year to accomplish a systematic survey, it cannot be assumed that the remaining counties are necessarily free from the disease. Evidence on this point will be adduced later in this paper.

The varieties of wheat on which perithecia of *Gibberella Saubinetii* were found are the following:—

Queen Wilhelmina	Diamant.
Red Marvel.	Squarehead's Master.
April Red.	

The first, Q. Wilhelmina, made up to 54 per cent. of the diseased specimens, but as this variety is more widely grown than any of the others, it was only natural that more of it would be received. At the same time, perithecial development on this variety appeared to be more prolific than on any of the others mentioned, and Q. Wilhelmina may be more susceptible to the disease.

The following data show previous cropping and treatment of wheat seed before sowing, in so far as details regarding these factors were supplied for crops diseased with *G. Saubinetii*:—

Crop preceding diseased wheat.		Seed treatment of wheat before sowing.	
Potatoes ...	59 per cent.	Agrosan	18 per cent.
Sugar Beet ...	9 per cent.	Ceresan	5 per cent.
Lea	9 per cent.	Mercurial dust unspecified ...	5 per cent.
Oats	9 per cent.	Copper Sulphate ...	9 per cent.
Wheat	6 per cent.	Tar and Lime ...	5 per cent.
No details ...	8 per cent.	No treatment ...	32 per cent.
		No information ...	26 per cent.

Losses due to the Disease.

As no information on reduction in yield of grain due to this disease is available in these islands, arrangements were accordingly made to obtain actual yields from some of the diseased fields. The following three examples are from the east coast, midlands, and south-west of the country, respectively. The variety grown in all three cases was Queen Wilhelmina, preceded by a root crop, viz., sugar beet in Co. Westmeath and potatoes in both the other counties.

Table I.—Yield of Grain from Crops attacked by *GIBBERELLA SAUBINETII.*

County.	Area under wheat. Statute acres.	Yield of grain. Stones.	Calculated yield per statute acre. Stones.
Co. Louth	0·8	100	125
Co. Westmeath ...	2·2	217	98½
Co. Kerry	1·8	130	72

In the case of the Co. Westmeath crop the bushel weight of grain was only 41 lb., whereas the merchants' range of bushel weights for the same district as that in which the outbreak occurred ranged from 48 lb. (one only) to 61 lb., with an average of 56 lb. In fact, so poor did this crop appear that the first visiting mill-owner declined to thresh it, as he charged on the barrel rate.

If 160 stone (one ton) be taken as an average yield per statute acre, a figure which is not excessive, then it is obvious that serious losses occurred, the reduction in yield of grain being 21·8, 38·4, and 55 per cent., respectively, in these three cases. The actual reduction in weight of grain does not, however, represent the total loss incurred, as an examination of 500 grains taken at random from each of these crops showed. In that from Co. Louth 22 per cent. were visibly affected by the fungus, the majority of them showing more or less pink discoloration; in the sample from Co. Westmeath discoloured grains totalled only 6 per cent., but shrivelled grains amounted to 44 per cent.; and in wheat from Co. Kerry 27 per cent. showed evidence of being infected.

Description of the Disease on Wheat.

The Disease on Wheat Ears.—While the fungus *G. Saubinetii* may attack any part of the wheat plant, it is that phase of the disease on the ears which causes the greatest loss. Infection of the ears may take place from the flowering period onwards throughout the time the grain is in the stooks. The most susceptible time, however, and that during which the worst damage is done, appears to be from flowering until the grain is in the soft dough or "setting" stage. Any part of the wheat head may be attacked, and usually a single spikelet is affected first. The earliest sign of infection is the appearance of a small brown or water-soaked spot on the outer glume, which soon bleaches, and under favourable climatic conditions the disease spreads to the entire spikelet. Affected areas dry out, take on a ripened appearance, and the diseased spikelets stand out in marked contrast to the healthy green spikelets. The glumes of an infected spikelet may remain closed, being held together by the fungus growth within, and a superficial pink growth of the fungus may be evident at the base of the glumes. Under moist conditions a complete weft of whitish or pink mycelium may develop over the entire spikelet (Fig. 1, Plate 4). Depending upon dry weather conditions, a single spikelet only may be affected, and the progress of the disease stopped by lack of moisture, but in wet seasons, as in 1942, the disease spreads to spikelets above and below the original point of infection, giving rise to a group of diseased spikelets. In numerous cases examined in 1942, the central portion of the head was the part most commonly attacked, although groups of infected spikelets also occurred on the upper and lower parts of the heads. Occasionally, the whole head was diseased, but more frequently a single group of diseased spikelets only was present. Very often, however, the injury to the whole head was out of all

proportion to the actual number of diseased spikelets. This was owing to the fungus invading the rachis of the head at the base of an early infected spikelet, this invasion stopping all water and food supplies to that part of the head above the point of attack. Heads, or portions of heads, which have been killed by early seasonal attacks in this way soon become bleached, and take on the appearance of premature ripening. Nevertheless, later in the year towards harvest, owing to weathering the same heads are darker in colour than the golden healthy ones. Furthermore, such badly diseased heads remain erect when healthy heads are turning downwards under the weight of ripening grain. The contrast towards harvest time between late infected spikelets and healthy ones in the same head is not so obvious as in the case of the early infections, the only evidence of disease as a rule being the presence of a pinkish mycelium at the base of diseased glumes during damp weather.

All investigators are agreed that the grains themselves are frequently invaded. Examination of affected heads after harvest in 1942 showed all stages of the disease to exist. In some, the whole head was much smaller than normal and many of the spikelets were completely barren, the remainder enclosing a few shrunken grains. In others, though grain was present in the majority of the spikelets, it was undeveloped and shrivelled (Fig. 9, Plate 5), and showed more or less pinkish discoloration, while a felty growth of mycelium was present on the surface of some of the grains. A third type had plump firm grain in the lower part of the head, but all above a certain point on the rachis was shrunken. Finally, in a minority of cases, all grains present in the head were apparently well developed, but a few showed reddish discoloration along the groove or at the embryo end. The foregoing are evidently typical results following the time infection of the head occurred; the first representing early infection at flowering, and the last late infection when grain was almost mature, the others being intermediate stages. Infection of individual grains varies from slight to that of complete permeation by the fungus and destruction of the embryo. All shrivelled grains in an infected head are not necessarily diseased, as this depends on their proximity to the point of infection on the rachis. On the other hand, normal-looking seed taken from a diseased head may be infected by the fungus, although no discoloration is visible.

The pink or reddish discoloration was very prominent on many samples of diseased grain in 1942, the colour being most intense at the germ-end, and varying from "rose pink" to "rose colour" according to Ridgway (24). Very often a pink colour was also present inside the glumes. Although a partially coloured caryopsis was the rule, one sample was received consisting of 160 threshed grains which were coloured all over, and had the appearance of having been dipped in red ink or an eosin solution. As these grains were well developed, and, as no complaint had been made by the correspondent about shrunken grains, this sample was probably the result of late infection occurring either in the stook or stack. Bennett (5) has pointed out the very favourable conditions existing in stooks for the spread of this disease, and the prolonged

harvesting operations in 1942 certainly provided the requisite factors for infection of healthy grain.

Conidial stage.—Microscopical examination of diseased grain and glumes showed that in some cases mycelium and conidia were rather sparse, but in others both were quite abundant. The conidia were of the *Fusarium* type, the number of septa varying from three to six, with five most common where conidia were numerous. Measurements of the conidia agreed in general with those recorded by Bennett (4) for conidia of *Gibberella Saubinetii*. The conidia serve for the rapid spread of the disease throughout the growing season, and, while the conidial stage of the fungus is the one that is most frequently observed, under certain climatic conditions in autumn perithecia containing asci and ascospores also develop on infected plants. These latter fructifications occurring on debris from diseased plants provide one of the methods by which the fungus is enabled to survive the winter.

Perithecial stage.—It has already been mentioned that wheat heads which have been killed by early seasonal attacks of *G. Saubinetii* are darker than healthy heads coming on to harvest, and in wet seasons blackening of wheat ears due to various moulds is not uncommon. Perithecia on infected heads, however, in 1942 presented an appearance totally unlike that of any other disease. At a casual glance the effect was as if a very black smut was being liberated from some of the flowers, and contaminating the surrounding group of spikelets (Fig. 2, Plate 4). This was the common picture of diseased heads. Closer investigation showed that the inky-black dots and blotches consisted of fructifications, either singly or so numerous as to form crusts on the outside of the spikelets (Figs. 3 and 4, Plate 5). Microscopical examination proved the perithecia to be those of *Gibberella Saubinetii* (Mont.) Sacc. They were produced chiefly on a plectenchymatic stroma which was usually well developed. The perithecia were mainly of the ovoid verrucose type, as described by Wollenweber (33) and by Bennett (4) (Fig. 5, Plate 5), though squat forms occurred. The majority of the perithecia were mature, and contained numerous eight-spored asci, the ascospores being four-celled as a rule, although occasional uniseptate ascospores were seen. The limits of height for mature perithecia were—min. 160μ, max. 300μ; and of width, min. 160μ, max. 251μ. As can be seen from these measurements there was great variation in size of the perithecia. Where they occurred singly, as in Fig. 3, Plate 5, perithecia were on the whole larger than when they were crowded together on the stroma. On bearded varieties of wheat, like April Red, perithecia developed along the awns as well as on the glumes.

Notwithstanding the profusion of perithecia during the year 1942, none occurred on the actual wheat caryopsis itself. Some attention was paid to this point, and not only was grain from spikelets covered with perithecial fructifications scrutinised, but many pounds of diseased grain were examined with negative results. In addition, suspicious wheat grains were received several times from Dr. Lafferty, of the Seed Testing Station, Dublin, and,

although on two occasions perithecia of *G. Saubinetii* were found on fragments of glumes in the sample, in all cases the black dots which were present on grain proved to be clusters of fungal hyphae and conidiophores, a species of *Alternaria* being common. In the case of wheat, therefore, the occurrence of perithecia on the actual caryopsis itself does not appear to take place under field conditions. It is true that Bennett (4) reported the formation of perithecia on wheat grains, but the conditions under which they developed were somewhat artificial.

GIBBERELLA SAUBINETII ON OATS.

Oat panicles, belonging to the varieties Marvellous and Victory II, bearing mature perithecia of *Gibberella Saubinetii* were forwarded by Mr. H. O'Donnell, Agricultural Instructor, Co. Galway. In the case of Victory II, Mr. O'Donnell reported that although he could find plenty of infected heads throughout the crop the disease on all of them was slight, and furthermore the disease was worst on that part of the field where lodging had occurred.

Owing to the open nature of the oat panicle, fungus infection seldom spreads to the central axis, therefore there is no cutting off of food supplies from healthy spikelets, and the disease is not nearly so destructive as in the case of wheat. In the specimens examined scattered infected spikelets were more common than groups of diseased spikelets, but occasionally several spikelets on a branch of the panicle showed infection as in Fig. 10, Plate 4. Nevertheless, even where infection was as bad as this on a branch of a panicle, the remainder of the head was frequently healthy.

The two samples referred to above were the only samples of infected oats received, but they constitute the first record of perithecial formation of *Gibberella Saubinetii* occurring naturally on oats in these islands. As perithecia were relatively abundant, it is probable that further investigation will prove the disease to be more widespread on oats than this single record indicates.

McInnes and Fogelman (19) reported oats to be only slightly susceptible to *G. Saubinetii*. This conclusion, however, may have been due to the particular strain of the fungus studied. Chih Tu (7) found certain strains of *G. Saubinetii* to attack oats worse than wheat; and in Finland, Rainio (23) reports oats as being badly infected.

CLIMATIC CONDITIONS AND DEVELOPMENT OF THE DISEASE IN 1942.

In County Dublin, that period of 1942 extending from April to September, inclusive, was not only the wettest, but had the least sunshine for any similar period during the past eleven years. Rainfall in May was over 4 inches, but the precipitation in June was only 0·12 inch, which was distributed over four days, hence the month of June was dry. This, however, was the only fine summer weather during the year. From the time wheat was flowering or just

setting its grain at the beginning of July, climatic conditions were bad, the weather being dull, cloudy, and wet, and as the season advanced they became worse. Similar weather occurred over the greater part of Eire, but with considerably more rain elsewhere, see Table 2. On the east coast the daily maximum shade temperatures for July varied from 64°–74° F. with a mean of 67·7° F.; in August 61°–74° F. with a mean of 66·7° F.; and in September 56°–73° F. with a mean of 63·6° F. The daily minimum shade temperatures for the same months were: July 43°–61° F., mean 52·4° F.; August 45°–60° F., mean 53·5° F.; and September 34°–56° F., mean 49·1° F.

The humid conditions existing throughout the greater part of the growing season evidently favoured the development of *G. Saubinetii*, both the conidial and perithecial stages being abundant. Judging from the epidemic of the

TABLE 2.

Number of days on which precipitation occurred, and rainfall for each month, April to September, inclusive, 1942, at Botanic Gardens, Glasnevin, Co. Dublin, and Agricultural Stations at Ballyhaise, Co. Cavan, Athenry, Co. Galway, Clonakilty, Co. Cork.

	April		May		June		July		August		September		Totals	
	No. of days	Rainfall inches	No. of days	Rainfall inches	No. of days	Rainfall inches	No. of days	Rainfall inches	No. of days	Rainfall inches	No. of days	Rainfall inches	No. of days	Rainfall inches
Glasnevin, Co. Dublin. (East coast)	13	1·12	18	4·20	4	0·12	19	2·14	22	3·76	18	4·48	94	15·82
Ballyhaise, Co. Cavan. (North midlands)	10	2·08	15	4·62	4	0·12	19	3·51	21	4·95	23	4·83	92	20·11
Athenry, Co. Galway. (West coast)	8	2·29	16	4·66	3	0·25	23	4·54	20	4·24	17	5·10	87	21 08
Clonakilty, Co. Cork (South coast)	16	1·95	21	4·75	4	0·51	16	2·69	17	4·36	19	2·92	93	17 18

disease and the abnormally cloudy wet weather, rainfall appeared to be the chief meteorological factor concerned, not only in the degree of infection and progress of the disease afterwards, as is borne out by all investigators, but also in the development of the perithecia. Dickson, Johann, and Wineland (11) have also suggested that perithecial development is closely associated with moisture.

EVIDENCE OF *GIBBERELLA SAUBINETII* BEING ENDEMIC IN EIRE PRIOR TO THE EPIDEMIC OF 1942.

Mention has already been made of the isolation of *G. Saubinetii* by Dr. Lafferty from wheat grown in Co. Galway in 1918, and some evidence will now be presented to show that the fungus has been endemic in certain districts for some time.

The Department of Agriculture received a sample of badly diseased wheat from Co. Clare in the month of August, 1930. When this wheat was examined by the Plant Pathological Division a *Fusarium* was found to be present, but the particular species was not identified. On carrying out feeding tests with

pigs and the diseased grain at that time, the Department of Agriculture found that such grain interfered with the normal development of the animals. Some of this wheat, which was kept by the Department of Agriculture, was re-examined by the writer in November, 1942, and many of the grains showed a carmine discoloration along the groove and at the embryo end. Attempts, however, during the past winter to isolate a fungus from the discoloured grains were all negative, the grains being sterile. This is not surprising, as Shands (28) found that in the case of barley grains affected with *G. Saubinetii* the fungus was not viable after 30 months, and Christensen and Kemkamp (8) report that plating tests of three-year-old diseased grains proved the fungus to be dead. The whole appearance of the diseased grain, toxic properties, and the presence of a *Fusarium* when first received, suggest that the fungus present originally was probably the conidial stage of *Gibberella Saubinetii.* Numerous diseased specimens were received from Co. Clare in 1942, and reports from both Mr. T. Healy and Mr. T. F. Murphy, Agricultural Instructors, show that the disease was widespread in the county during the past season. Mr. Healy reported that he had observed the disease several times since 1930; and Mr. Murphy stated that infection was very noticeable on the vast majority of crops inspected in west Clare in 1942, the disease being prevalent on February sowings of winter varieties of wheat.

Reports to hand from other counties also indicate that the disease is not by any means new. In Co. Galway (where the fungus was found twenty-four years ago) Mr. H. O'Donnell, Agricultural Instructor, reported that he could find traces of the disease in almost every haggard visited during the past year. The disease has been under the observation of Mr. M. Hession, Agricultural Instructor, Co. Sligo, for the past 10–12 years, but he writes that it never was so bad as in 1942. The foregoing three counties are all on the western seaboard, but the disease has not been absent from the east coast. Mr. R. McIvor, Agricultural Instructor, Co. Louth, states, that in the case referred to in Table 1, the farmer noticed the disease three years ago, a small sheltered portion of the crop being then affected.

All observations on growing crops previous to 1942 refer of course only to a *Fusarium* stage, which may have included several species. While this may be so, it is very unlikely that *Gibberella Saubinetii* was absent and then in 1942 became the predominant fungus on wheat heads, and it is more reasonable to assume that the fungus has been endemic in certain districts for a long time.

Observations from the Seed Testing Station, Department of Agriculture, Dublin.

To begin with, it should be understood that these observations have been made quite independently of the author's own investigations. They are included here with the permission and approval of Dr. H. A. Lafferty, Head of the Seed Testing Station, to whom the writer is deeply indebted both for the

figures given herewith and for many samples of diseased grain during the past season.

After the harvest of 1938 (which incidentally followed a wet summer) attention began to be paid to a red fungus which was occurring on wheat samples undergoing germination tests in the Seed Testing Station. It is probable that the fungus occurred previously to 1938, but was not sufficiently prevalent on samples to attract undue notice. Apart from identifying the fungus as a *Fusarium* spp. no further investigation of it was undertaken except to record it on the germination sheets as the "Red Disease" on all samples where it occurred. Table 3 shows the percentages of so-called "Red Disease" recorded since the harvest of 1938 until the time of this paper going to press.

TABLE 3.

Year.	No. of Wheat Samples tested.	No. showing "Red Disease."	Percentage of Samples showing "Red Disease."
1938/'39 ...	1874	12	0·6
1939/'40 ...	5261	146	2·7
1940/'41 ...	5658	140	2·4
1941/'42 ...	7347	173	2·3
1942/'43 ...	4863	1232	25·3

The first year in each case is the harvest year.

It is obvious from Table 3 that there was an exceptional development of the "Red Disease" on wheat crops last year. Since September, 1942, samples of wheat with this disease have been received from all of the 26 counties in Eire. In some cases 50 affected samples have come from a single county. The figures in Table 3 do not give any indication of the severity of the disease on the samples, but simply show its prevalence. Some of the samples of grain had only 1 or 2 per cent. of the disease present, but others were extremely bad. During the past winter severely infected samples showed as low germination as 29 and 33 per cent.

All of the infections shown in Table 3 are not necessarily caused by the fungus *Gibberella Saubinetii*, but, as it has been found repeatedly on wheat samples received recently from the Seed Testing Station, the present indications are that it is one of the principal species responsible for the so-called "Red Disease."

"SEEDLING BLIGHT."

Blight of cereal seedlings due to *Gibberella Saubinetii* has not yet been reported in this country, and to what extent this fungus may be involved in causing thin stands of wheat is not at present known.[2] That *G. Saubinetii*

[2] Since this paper was sent to the printers a number of cases of "Seedling blight" due to *Gibberella Saubinetii* have already come to hand from several counties. Practically all these cases have been on February sowings of the variety Queen Wilhelmina.

attacks wheat seedlings readily and extensively has been proved many times, and amongst the numerous investigators who have studied this problem the following consider this fungus to be a virulent parasite on wheat roots:—Adams (1), Atanasoff (2), McInnes and Fogelman (19), Dickson (10), and Henry (16). Bennett (5) also reports the fungus as capable of attacking the root system. "Seedling blight" may develop either from the fungus present in contaminated soil or from infected grains, but the latter appears to be the more common origin. Atanasoff (2) points out that an important source of infection is the seed used for sowing; and Nelson (21) states that after the wet season of 1928 many farmers in Michigan, U.S.A., were obliged to use scabbed seed, and a considerable amount of seedling blight occurred in 1929.

The symptoms of this phase of the disease depend upon the part of the seedlings attacked and the environmental conditions, Dickson (10). Thin brairds may result from sowing naturally infected seed, some of which may not be viable at all depending upon the degree of infection, or many of the germinating seedlings may be killed before they appear above ground. In less severe attacks seedlings emerge, but are weak and chlorotic, and they may succumb up to the development of the second leaf. Browning and rotting of the roots occur, the fungus mainly attacking and destroying the central cylinder first and the cortex afterwards, according to Henry (16). Congested growth of the fungus also takes place in the crown of the plant, and this, together with reduced root system, causes the death of seedlings, Bennett (5). Beyond the second leaf stage many affected plants apparently recover and produce normal heads of grain. However, depending upon the intensity of basal invasion and environmental conditions, affected plants may develop more or less "foot rot," dwarfing, and premature ripening, Bennett (5).

According to Dickson (10) the temperature of the soil is the most important single factor determining the extent of "seedling blight." For the development of the blighting of wheat he found the most favourable soil temperatures to range from 12° to 20° C. Gäumann (15) has also shown that temperature is an important factor in the invasion of wheat seedlings by *G. Saubinetii*. While a relatively high temperature is favourable for the disease, Dickson (10) found that low soil moistures accompanying low temperatures also favoured its development. That infection can take place at low temperatures is likewise shown by Bennett's investigations; he found that wheat seedlings became infected when the temperature of the greenhouse did not exceed 10° C. Furthermore, Bennett reports that "seedling blight" developed worst under conditions adverse for the host, i.e. insufficient soil moisture or water-logging.

The fungus grows up the stems of affected plants for some distance, but it is not considered systemic by the majority of investigators. Infection of the ears is derived mainly from wind-borne conidia or ascospores alighting on the anthers and glumes. The occurrence of diseased spikelets in groups at various parts of the head with healthy intervening spikelets indicates aerial infection rather than internal mycelium.

Summary of Our Knowledge on the Toxic Properties of Diseased Grain.

As the utilization of diseased grain is always an important economic question, and especially so under the present war-time conditions and scarcity of feeding stuffs, it is considered desirable to include the following summary in the present paper.

Amongst the numerous fungi which attack cereal heads *Gibberella Saubinetii* is the most serious of those producing toxic substances in affected grain. The injurious substances are transmitted to the manufactured product, and symptoms of poisoning and intoxication follow the consumption of bread made from such grain. The disease has long been familiar to Chinese farmers in Eastern Siberia, and the so-called "inebriating" bread is well known in various parts of the Russian Empire, Shapovalov (27), Dounin (13).

Following an epidemic of *Gibberella Saubinetii* in North America in 1928, and the subsequent importation of large quantities of diseased barley into Europe, severe poisoning of pigs occurred over considerable districts in Northern Germany and in parts of Holland. This led to intensive studies on this problem both in Europe and America.

Roche, Bohstedt, and Dickson (25) report that pigs, horses, and dogs, as well as man, are very sensitive to low percentages of scabbed grain, but that infected barley may be fed economically to cattle, sheep, and poultry. Mains, Vestal, and Curtis (18) found that diseased barley may be given to hogs when grain does not exceed 10 per cent. of the total ration. Barley containing up to 58 per cent. of scab was successfully fed to cattle as 50 per cent. of the grain ration, and was utilizable to the extent of 20 per cent. of the poultry ration. Mundkur and Cochran (20) reported extensive poisoning of hogs and poultry in Iowa by consumption of barley affected with *Gibberella Saubinetii* where perithecia were present on the surface of 4 to 8 per cent. of the grain. In feeding tests they found that the effects of diseased grain were variable on poultry. Young chickens fed on scabby grain developed rough plumage and lost weight, but mature birds showed no ill effects. That poultry can tolerate considerable amounts of diseased grain is also shown by the work of Titus and Godfrey (31). Rainio (23) in Finland found oats infected by *G. Saubinetii* injurious to pigs and horses when infection exceeded 20 per cent. of the grain; between 10 and 20 per cent. infection, the food was unpalatable or eaten reluctantly with consequent digestive troubles. Cattle showed no loss of appetite unless the infection exceeded 20 per cent. Christensen and Kemkamp (8) proved that scabbed wheat when fed to pigs caused the same harmful effects as similar quantities of scabbed barley. These two authors give a good clinical description of affected animals, and state that with 10 per cent. of the grains diseased the pigs did not thrive, and that barley naturally infected with 16 per cent. scab was extremely toxic to pigs.

Beller and Wedemann (3) believed the noxious principle to reside in certain organic compounds which are not susceptible to chemical analysis. Dickson *et al.* (12) considered the active substances to be apparently associated with factors containing glucosides or basic nitrogen compounds. The investigations of Schroeter and Strassberger (26) indicated that the symptoms of poisoning in animals are induced by cholin or readily hydrolysable fatty acid esters of cholin. Popp (22) attributed the poisoning properties to chemical changes in composition of the barley, the carbohydrates and albumins being partially disintegrated and toxalbumin or related toxic nitrogenous compounds formed, which caused sickness in pigs and also in poultry. Hoyman (17) found that extracted toxic substances were resistant to drying and partly inactivated by autoclaving: analysis indicated the presence of nitrogen but no sulphur or halogens.

The toxic properties of diseased grain may be reduced somewhat by prolonged storage with repeated shovellings, or by soaking and washing with water. In general the work of these investigators has shown that grain badly infected with *Gibberella Saubinetii* is not a satisfactory food for pigs and horses. It may, however, be fed to cattle, sheep, and poultry when mixed with other grains.

Discussion.

The fungus *Gibberella Saubinetii* (Mont.) Sacc. has been reported from Europe, North and South America, Asia, Africa, and Australia. It attacks plants belonging to many genera and widely separated families (2), but it is most destructive on the Gramineae, cultivated cereals suffering severely. The fungus is apparently co-existent with wheat throughout its range of cultivation. In many countries the disease is relatively unimportant, but in others it is endemic in certain districts, giving rise to regular epidemics when climatic conditions are favourable.

The common name of the disease, "Ear Blight," as used in these islands, is a designation applied to several species of *Fusarium*. In America, "Wheat Scab" (1, 19, 21), "Fusarium-Blight" (2), and "Headblight" (7) are all in common use. I have been unable to ascertain when, or by whom, the term "scab" was first given to the disease on wheat. McInnes and Fogelman (19) state that Smith in 1884 named it "Wheat Scab." This statement, however, is not correct, as an examination of Smith's book (30) shows no mention of scab, although he apparently first described the blighting of wheat heads. Atanasoff (2), in his remarks on the common name of the disease, points out that in America "the head-blighting of cereal crops is generally known under the faulty name of "wheatscab"; and he goes on to say, "It is not a wheat disease alone, because it also occurs on spelt, rye, barley, oats, and certain grasses. And it is not 'scab' because it causes no scabbing of the heads or any part of the various hosts, but rather blighting of the heads." With this

last sentence the present writer is not in agreement. The perithecial stage of the fungus as it occurred in Eire in 1942 consisted of definite incrustations on the spikelets, giving them a scabbed appearance, Fig. 2, Plate 4, and Figs. 3 and 4, Plate 5, and "Wheat Scab" or—as the disease occurs on other cereals—preferably "Cereal Scab" is a very appropriate name for this phase of the disease.

Perithecial formation on the heads of both wheat and oats is so distinctive that it is rather remarkable there is no previous record of its occurrence in this country. It might be argued that owing to the present demands for extensive cereal cultivation, a much larger acreage is now under these crops than formerly, and hence, any disease would be more readily noticed. While this is partly true, wheat was grown in the past to some extent every year, and considerable areas of oats and barley have always been cultivated in this country. Granting that perithecia do not develop every year, wet seasons have not been infrequent, yet there is no account of anything resembling perithecial formation on any cereal crop. In the case of barley in particular, any outbreak of *G. Saubinetii* on the grain, either with or without perithecia, could scarcely have been overlooked, as the presence of the fungus on this cereal is stated to interfere with the malting quality, Shands (29). The epidemic on wheat in 1942 may have been due to a particular strain of the fungus which attacks wheat more readily than other cereals, but this point requires further investigation. Chih Tu (7) has shown that different strains of *G. Saubinetii* exist, varying in their virulence on wheat, barley, and oats, and also in their capacity to attack different varieties of the same host. None of the hosts mentioned, however, were immune to attack by any of the strains isolated. The pathogenicity of strains was also investigated by Ullstrup (32) and by Eide (14), the latter reporting difference in ability of strains to produce perithecia.

Owing to the fact that perithecia develop abundantly on stubble of affected crops, as well as on the ears, in countries where the disease is prevalent rotation of cereal crops is strongly recommended as one of the control measures. As wheat is just now being grown occasionally two or more years in succession, and under the present exigency frequently follows other cereals, it was thought that this might have been a predisposing factor in the epidemic of 1942. There is no evidence, however, that such was the case. From the data given, it is apparent that the disease was prevalent on wheat following root crops, occurring after roots in 68 per cent. of the cases reported, and in 59 per cent. of the cases potatoes constituted the previous crop. *Gibberella Saubinetii* is not known to occur as a regular parasite of potatoes, although Wollenweber (33) records isolating this fungus from potato berries. The severity of the disease on wheat sown after root crops was probably associated with the more luxuriant foliage of the wheat after roots rather than to any infection arising from the previous crop. Atanasoff (2) found that on healthy, vigorous, and more succulent plants the infection spreads more rapidly than on plants of average vigour.

Wheat from badly affected crops should not be used for seed purposes. It is evident that the germination of such seed will be low to begin with; secondly, its use may result in "seedling blight," or diseased plants may serve as a source of infection to the crop in general; and thirdly, it is clear from data given that seed dressings do not control this disease effectively.

The author is indebted to the Department of Agriculture, Dublin, for every facility in carrying out these investigations, and also to the various County Instructors in Agriculture who not only forwarded specimens for examination but unreservedly supplied many observations on the disease. Grateful acknowledgement is also made to the Imperial Mycological Institute, Kew, for the loan of several Bulletins which were unobtainable in Eire.

Summary.

An outbreak of *Gibberella Saubinetii* (Mont.) Sacc. on an epidemic scale on wheat in Eire in 1942 is described.

The disease appeared during the flowering period of the host, and in some cases was so severe that many wheat heads had no fully developed grains, the flowers either being empty or having shrivelled seed. From 0·8, 2·2 and 1·8 statute acres the actual yields of grain were 100, 217 and 130 stones, respectively. Reduction in yield due to the disease in these cases was of the following order:—21, 38, and 55 per cent. These figures, however, do not represent the total loss, as much of the remainder of the crop in each case consisted of shrivelled grain of low germination.

The epidemic was associated with showery weather which began early in July, and as the season advanced climatic conditions became worse. The summer of 1942 had the least sun and was the wettest in Eire for eleven years.

Perithecial development occurred freely on several varieties of wheat and was recorded from ten separate counties. It is believed that moisture rather than temperature was the main factor responsible for perithecial formation.

Perithecia of *G. Saubinetii* were also found on two varieties of oats. This apparently constitutes a first record of these fructifications occurring naturally on oats in these islands.

Some evidence is presented to show that the disease has been endemic in certain districts for a considerable time.

Owing to the scabbed appearance of spikelets of wheat and oats with perithecia of *G. Saubinetii* on them, "Cereal Scab" is quite an appropriate name for this phase of the disease.

EXPLANATION OF PLATES.

Plate 4.

Fig. 1.—Healthy wheat head on left, two diseased heads on right showing general effects of attack and weft of mycelium of *G. Saubinetii* on affected spikelets. Variety Queen Wilhelmina.

Fig. 2.—Perithecia of *G. Saubinetii* on wheat ears, affected spikelets occur in groups. Variety Q. Wilhelmina.

Fig. 10.—Branch of oat panicle showing perithecia of *G. Saubinetii* on two spikelets. × 3.

Plate 5.

Figs. 3 and 4.—Spikelets removed from diseased wheat heads, showing medium and prolific development of perithecia of *G. Saubinetii*. × 3.

Figs. 5 to 8.—Photomicrographs. Fig. 5.—Median longitudinal section through perithecium of *G. Saubinetii* showing general appearance. × 200. Fig. 6.—Section through perithecium but not median, showing proliferation of cells around the neck of the fructification. Section squashed to show numerous asci and paraphyses. × 200.

Fig. 7.—Mature ascospores in ascus. × 750.

Fig. 8.—General appearance of ascospores when removed from ascus. × 1000.

Fig. 9.—Wheat grains from affected ears on left, healthy grains on right.

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FIG. 1.

FIG. 2.

FIG. 10.

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FIG. 3.

FIG. 4.

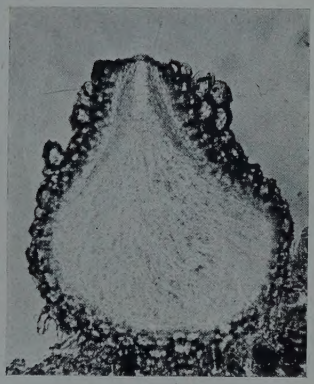

FIG. 5.

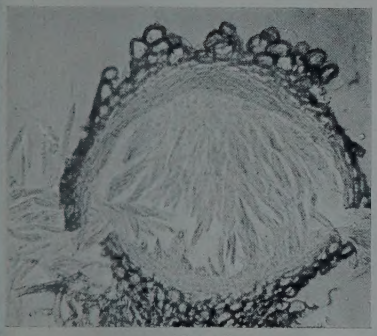

FIG. 6.

FIG. 7.

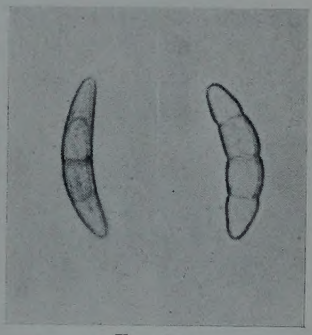

FIG. 8.

FIG. 9.

Photos] [*G. H. McLean.*

SCIENTIFIC PROCEEDINGS.

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1. Report of the Irish Radium Committee for the Year 1941. Including Reports from OSWALD J. MURPHY, M.B., B.CH., St. Vincent's Hospital, Dublin; ST. ANNE'S HOSPITAL, DUBLIN (*Radiologist*—Dr. Oliver Chance); R. E. TOTTENHAM, M.D., B.CH., B.A.O., City and County Hospital, Londonderry; and OLIVER CHANCE, M.B., F.F.R., D.M.R.E., Richmond, Sir Patrick Dun's, Dr. Steevens', Rotunda, and Coombe Hospitals. (July, 1942.)

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